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Research article

Dealing with Ebola virus disease in Spain: epidemiological inquiries received by the Department of Public Health Alerts, April to December 2014

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We describe the inquiries regarding Ebola virus disease (EVD) received by the Department of Public Health Alerts of the Community of Madrid between April and December 2014. A total of 242 inquiries were received. Consultations were initiated most frequently by hospital clinicians (59 inquiries, 24%), private citizens (57 inquiries, 24%) and primary care physicians (53 inquiries, 22%). The most frequent topic of inquiry was possible EVD in a patient (215 inquiries, 89%). Among these, 31 persons (14%) presented both EVD-compatible symptoms and epidemiological risk factors, and 11 persons (5%) fulfilled the criteria for a person under investigation. Recent travel abroad was reported in 96 persons (45%), but only 32 (15%) had travelled to an EVD-affected area. Two high-risk and one low-risk contact were identified through these inquiries. Low specificity of the EVD symptoms led to many difficulties in protocol application. Ineffective communication with healthcare professionals and unfamiliarity with the EVD protocols caused many case classification errors. A rapid consultation service by telephone is essential for providing qualified advice during emergencies. Our experience may help other countries dimension their activities and resources for managing similar exceptional outbreaks in the future.

Introduction

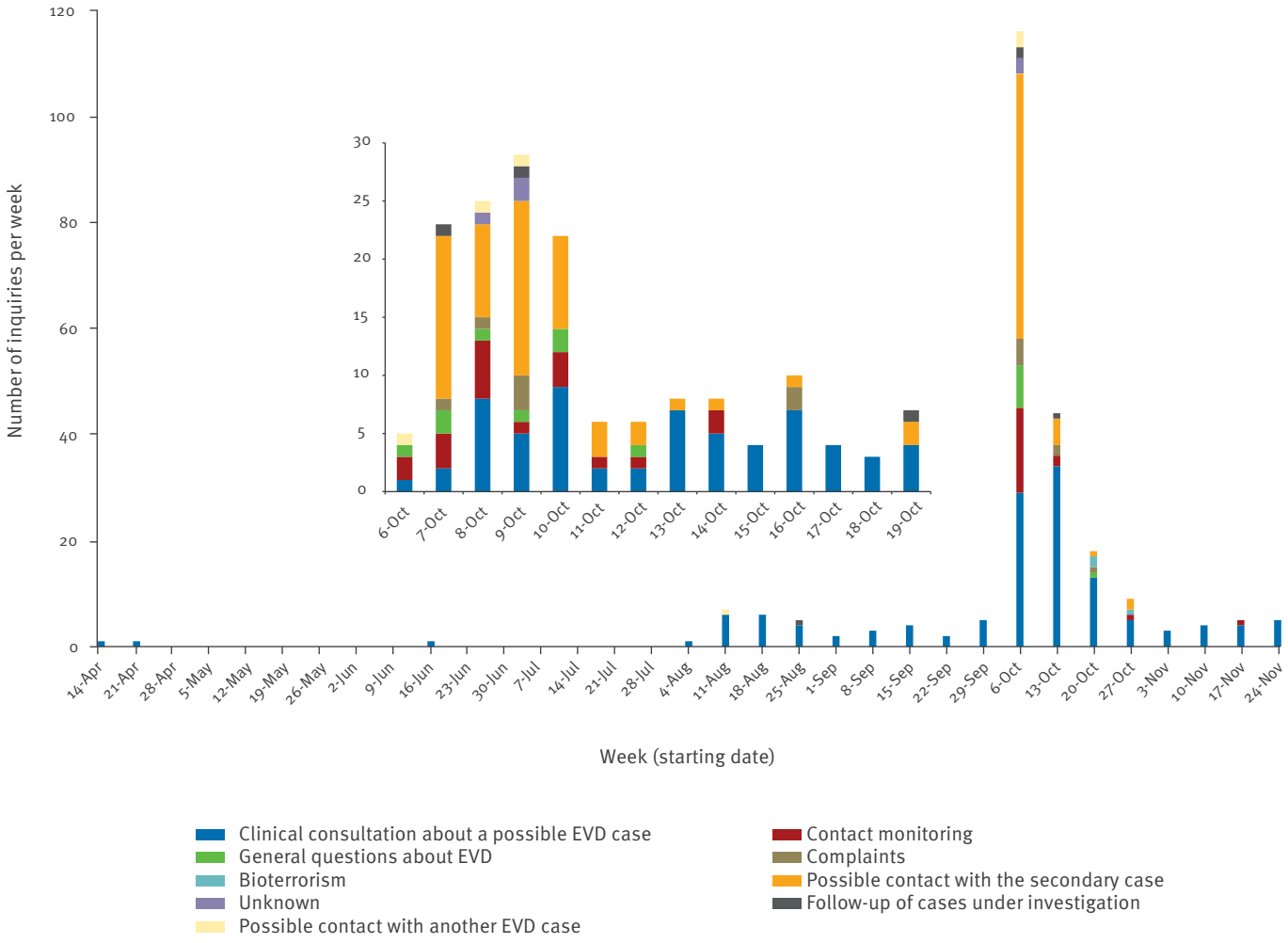
The ongoing outbreak of Ebola virus disease (EVD) in West Africa is the largest registered outbreak of this disease in history. Liberia, Sierra Leone and Guinea have been affected the most, with more than 27,000 cases and over 11,000 deaths between December 2013 and June 2015 [1]. Isolated imported cases or small outbreaks with secondary transmission of EVD have also been reported from Nigeria, Senegal, Spain, the United States (US), Mali, the United Kingdom and Italy [2-8].

The World Health Organization first announced the EVD outbreak at the end of March 2014 [9], and the Spanish Ministry of Health, Social Services and Equality (MoH) issued the initial Ebola virus public health warning on 1 April 2014 [10]. On 7 August 2014, the Spanish government decided to repatriate a Spanish healthcare worker from Monrovia (Liberia), who had tested positive for the Ebola virus. The missionary was admitted to the La Paz-Carlos III Hospital Complex, a designated reference centre for management of infectious diseases, but died on 11 August. On 22 September, a second Spanish healthcare worker who was also suffering from EVD was repatriated from Sierra Leone and admitted to the same reference hospital, where he died on 25 September. On 6 October, the Spanish National Reference Laboratory confirmed the first human-to-human transmission of EVD outside of Africa in one of the healthcare workers who provided care for the second repatriate [4,11,12].

Spain is administratively divided into 17 Autonomous Communities which have their own healthcare and public health systems; the role of the MoH is to act on interregional, national and international level. The Community of Madrid has particular experience in the management of public health threats of international importance given the presence of an international airport and the aforementioned La Paz-Carlos III Hospital Complex. After the arrival of the first repatriate, the Community of Madrid activated its International Alert Management Protocol and an Ebola Coordination Centre, led by the Department of Public Health Alerts of the Community of Madrid (the Department). The objective of this study was to describe the EVD-related inquiries received by the Department between 1 April and 2 December 2014, when the Spanish Ebola outbreak was officially declared to be over [13].

Figure 1

Inquiries related to Ebola virus disease received by the Department of Public Health Alerts, Community of Madrid, Spain, 1 April–2 December 2014 (n = 242)



EVD: Ebola virus disease.

Methods

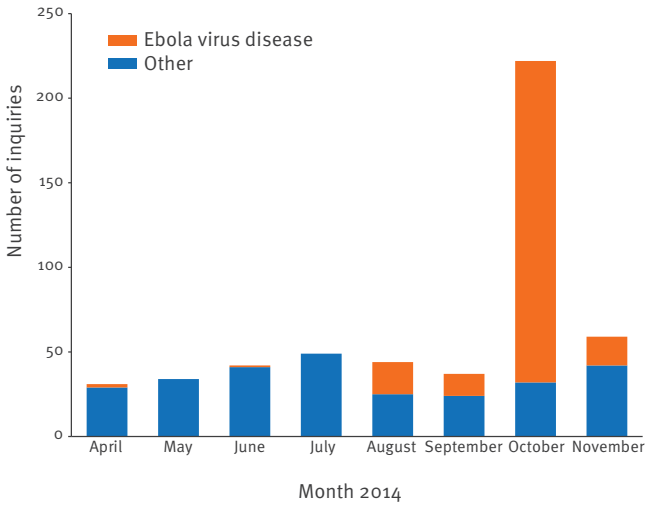
The Department is in charge of coordinating the response to the public health alerts in the Community of Madrid. Depending on the time of day, the EVD alerts are received either by the staff of the Department (office hours 08:00–15:30) or by an on-call public health officer through the Rapid Public Health Alert System (Sistema de Alerta Rápida en Salud Pública, SARSP), created by the Department in 2003 in response to the SARS epidemic (weekdays 15:30–08:00, weekends and holidays).

The Ebola Coordination Centre is formed by the Department, the SARSP and the Madrid Medical Emergency Service (SUMMA 112). The principal activities of the Ebola Coordination Centre are: classification of patients according to the epidemiological criteria, declaring the person as under investigation for EVD, ordering collection of a sample for PCR analysis, coordination of the transport of the samples to the National Reference Laboratory for Ebola virus testing, activation of the transport of persons under investigation or confirmed cases to the reference hospital, initial epidemiological survey of the patient, technical advice to healthcare professionals regarding the protocols, and answering the questions of contacts of the EVD cases.

The EVD alerts and EVD-related inquiries are reported via one of the three following paths: (i) The Border Health Control physician reports a person under investigation [14,15] directly to the Department or SARSP who activate the alert protocol and transport the patient to the designated hospital (La Paz-Carlos III Hospital Complex); (ii) Persons who present symptoms compatible with EVD and call the free emergency telephone number 112 are transferred to the medical coordinators of SUMMA 112 who carry out the initial evaluation and report the person to the Department or SARSP for further epidemiological evaluation; (iii) Primary care or hospital clinicians report their suspicion of a patient

Figure 2

Monthly inquiries received by the Department of Public Health Alerts. Community of Madrid, Spain, 1 April–2 December 2014 (n = 518)



that could have EVD through a free telephone number 061 to the medical coordinators of SUMMA 112, who forward the alert to the Department or SARSP.

All inquiries to the Department have therefore been previously evaluated either by a physician or by the emergency services staff. This study includes all EVD-related inquiries received by the Department in the period from 1 April to 2 December 2014. The data were extracted from the database of public health alerts, which is part of the Public Health Information System (Sistema de Información de Salud Pública, SISPAL), and completed with information from SUMMA 112 call logs and public health officers' notes. Information collected for the purpose of the present study comprised the date of the inquiry, notifier, topic of the inquiry, age and sex of the person concerned, presence of symptoms, recent travel abroad including travel dates and country visited, fulfilment of clinical and epidemiological case criteria, monitoring and results of the Ebola virus PCR.

The criteria that had to be met for declaring a person as under investigation for EVD are summarised in the Box [14]:

Results

Between 1 April 2014 and 2 December 2014, 242 telephone inquiries related to EVD were received in the Department (Figure 1). The proportion of EVD-related inquiries in relation to the total number of monthly inquiries to the Department is represented in Figure 2. Three additional epidemiologists and one administrative worker were hired for a period of three months to help deal with the workload related to the EVD outbreak.

Inquiries originated most frequently from clinicians: hospital clinicians initiated 59 (24%) and primary care physicians 53 inquiries (22%). Private citizens made 57 inquiries (24%, Table 1). The most common topic was possible EVD in a patient (133 inquiries, 55%), followed by concerns about possible contact with the secondary EVD case (58 inquiries, 24%, Table 1). Eight inquiries (3%) were complaints related to the management of the EVD outbreak and three calls (1%) were alerts about possible bioterrorist attacks: two separate incidents of envelopes containing a piece of red-stained fabric and marked as 'Ebola'.

Of all inquiries, 215 (89%) were clinical inquiries that concerned a possible case of EVD (133 clinical consultations about possible EVD in a patient, 62 concerns about possible contact with an EVD case and 20 consultations related to contact monitoring). Information about sex was available for 208 subjects (97%): 115 were men (55%). The mean age was 37.3 years (standard deviation: 15.1; range: 0–86; information available for 66% of the persons). In total 158 calls were about persons who had some symptoms consistent with EVD (73%; Table 2), but only 31 (14%) fulfilled strictly the clinical criteria of a person under investigation [14,15].

The most common EVD-compatible symptoms were fever or dysthermia, present in 124 cases (78% of persons with symptoms; Table 3). The epidemiological criterion was fulfilled in 54 persons (25%). Eleven cases (5%) fulfilled both criteria; four of these 11 cases were tested for Ebola virus, the remaining seven cases were not tested because alternative diagnosis or clarifications on the patient's history were obtained or because symptoms resolved before a blood sample for PCR was taken (the sample collection had to be approved in advance by the Department in order to coordinate the sample transport to the reference laboratory, which led to some delays). Another 11 cases (5%) were tested for EVD although they fulfilled only one of the two criteria: four of them were travellers from EVD-affected countries exhibiting some EVD-compatible symptoms and seven were healthcare workers who had had professional contact with an EVD case and presented low-grade fever that did not reach the established threshold; one of them was the secondary EVD case. In total, 15 cases were tested and all results were negative except for the secondary EVD case. An alternative diagnosis was available for 30 cases, the most common being malaria (12 cases, 8% of symptomatic persons) and traveller's diarrhoea (three patients, 2%).

Ninety-six inquiries (45% of the clinical inquiries) were related to reported recent travel abroad (less than 21 days before the onset of symptoms) and the callers were mainly physicians (80 consultations, 83%). Nine inquiries were initiated by private citizens (9%) and seven by other authorities (7%). The inquiries were most frequently related to travels to Nigeria (23 inquiries, 24%) and Equatorial Guinea (16 inquiries, 17%). Only 32 consultations regarding travellers involved a

Box

Definition of person under investigation for Ebola virus disease, Spain, 1 April–2 December 2014

Epidemiologic criteria – at least one of the following expositions in the previous 21 days:

- travel to an area with EVD transmission,
- contact with an EVD case (under investigation or confirmed) or with their body fluids or biological samples.

Clinical criteria:

- fever of › 38.6 °C and any of the following symptoms: intense headache, vomiting, diarrhoea, abdominal pain, any unexplained haemorrhagic manifestation or multiple organ failure,
- sudden and unexplained death.

After the diagnosis of the secondary EVD case, the fever threshold was decreased to ≥ 37.7 °C and the criteria for EVD contacts under surveillance were changed to the presence of increased body temperature and/or EVD-compatible symptoms [15].

history of recent travel to an EVD-affected area (33%; Table 4).

Discussion

Our study describes 242 EVD-related inquiries received at the Department during the EVD epidemic in 2014. All of these were highly specialised requests, previously triaged by SUMMA 112.

Four distinct phases may be observed in our study. During the first period, from the issue of an international EVD alert on 1 April 2014 to the repatriation of the first healthcare worker on 7 August, only three inquiries were received. The second period between 8 August and the date of diagnosis of the first autochthonous case of EVD in Spain on 6 October was characterised mainly by inquiries related to travellers arriving to Spain from African countries. Noticeably, none of the inquiries during this period were related to contact monitoring of the healthcare workers caring for the repatriates, probably because self-monitoring only was recommended when no breach of the protocol for using the personal protective equipment was reported [11,12,14]. The diagnosis of EVD in the healthcare worker on 6 October marked the beginning of the third period of what may be called a public health crisis. In the first hours and days after the information was published, the official communications were limited because the public health authorities were still conducting an investigation into the mode of transmission and tracing contacts [12]. For a few days, the media became the main source of updated information [16,17] and their constant and overwhelming focus on the case contributed to a panic in the population reflected in the peak of inquiries in the second and third week of October. Eventually, the government adopted a set of measures to improve the communication with the public (establishing a national Special Committee on Ebola Management, a webpage and a twitter account), all contacts were traced and controlled, the secondary case recovered. In this fourth period, the focus of the consultations turned back to travellers. However, notwithstanding certain deficiencies in the risk communication on behalf of the authorities, a disease as contagious and lethal as EVD encountered outside of its natural environment will inevitably cause social alarm and raise a wave of questions, fears and insecurities in the community. Similar evolution of EVD-related inquiries before, during and after a diagnosis of a cluster of three EVD cases in the US [18,19] was reported at the Centers for Disease Control and Prevention (CDC) [20].

Throughout the study period, we experienced various difficulties with the application of the EVD protocol. Before the diagnosis of the autochthonous EVD case, the expected route of introduction of EVD to the country was through travellers arriving to Europe from West Africa [21], and the first Spanish national EVD protocol focused on this scenario [14]. Medical evacuation of EVD cases was treated in a separate protocol [22] and was not a priori considered risky because operations were supposed to happen under the strictest infection control measures. Application of the case criteria in this period was rigorous, but even then, it was not as straightforward as one may expect. Many of the consulted cases in this period, for example, were African migrants returning from summer vacation in their homeland via Lagos international airport. The only affected states in Nigeria were Lagos and Rivers, but most of the consulted cases had stayed in other areas or even in other countries and only spent a few hours in Lagos at the airport on their way back, so the probability of a sustained contact with a symptomatic EVD case was very low. Because of the low specificity of the EVD symptoms, it was often difficult for the public health officers to decide whether to activate the EVD protocol, which would mean an admission to the reference hospital under strict isolation measures for several days, especially when other diagnoses such as malaria were much more likely [23]. This was probably taken into consideration when defining the epidemiological criteria during the outbreak in Mali in November

Table 1

Topic of inquiries related to the Ebola virus disease and alert notifier, Community of Madrid, Spain, 1 April–2 December 2014 (n = 242)

Notifier	Inquiry topic n (%)									
	Clinical consultation about a possible Ebola virus disease case	Possible contact with the secondary case	Possible contact with another Ebola virus disease case	Contact monitoring	Follow-up of cases under investigation	General questions about Ebola virus disease	Complaints	Bioterrorism	Unknown	Total
Emergency services	24 (18)	2 (3)	0	1 (5)	0	0	1 (13)	1 (33)	0	29 (12)
Primary Care	42 (32)	5 (9)	0	0	1 (25)	2 (22)	1 (13)	0	2 (67)	53 (22)
Hospital	43 (32)	9 (16)	0	4 (20)	2 (50)	1 (11)	0	0	0	59 (24)
Occupational Health Department	2 (2)	2 (3)	0	8 (40)	0	1 (11)	0	0	0	13 (5)
Border Health Control	5 (4)	0	0	1 (5)	0	0	0	0	0	6 (2)
Private citizen	8 (6)	33 (57)	4 (100)	3 (15)	1 (25)	2 (22)	4 (50)	1 (33)	1 (33)	57 (24)
Other/Unknown	9 (7)	7 (12)	0	3 (15)	0	3 (33)	2 (25)	1 (33)	0	25 (10)
Total	**133 (100) {55}**	**58 (100) {24}**	**4 (100) {2}**	**20 (100) {8}**	**4 (100) {2}**	**9 (100) {4}**	**8 (100) {3}**	**3 (100) {1}**	**3 (100) {1}**	**242 (100) {100}**

() Percentage in column. {} Percentage in row.

Table 2

Characteristics and management of cases handled via the Ebola virus disease consultation, Community of Madrid, Spain, 1 April–2 December 2014 (n = 215)

Topic of the consultation	n (% of the total of clinical consultations)					
	Symptoms[a]	Clinical criterion	Epidemiological criterion	Symptoms[a] and epidemiological criterion	Clinical and epidemiological criterion	PCR
Clinical consultation about a possible Ebola virus disease case (n = 133)	114 (53)	22 (10)	33 (15)	28 (13)	7 (3)	7 (3)
Possible contact with the secondary case (n = 58)	27 (13)	4 (2)	4 (2)	3 (1)	0	0
Possible contact with another Ebola virus disease case (n = 4)	3 (1)	1 (0)	0	0	0	0
Contact monitoring (n = 20)	14 (7)	4 (2)	31 (14)	12 (6)	4 (2)	8 (4)
Total (n = 215)[b]	**158 (73)**	**31 (14)**	**54 (25)**	**43 (20)**	**11 (5)**	**15 (7)**

[a] Symptoms compatible with Ebola virus disease: fever (or dysthermia), headache, vomiting, diarrhoea, abdominal pain, unexplained haemorrhagic manifestations, multiple organ failure, sudden and unexplained death.

[b] Some cases may be represented in more than one column.

to December 2014: passing through the Bamako International Airport only was excluded. On the other hand, it was difficult to strictly adhere to the body temperature criterion in persons returning from countries with intense EVD transmission and release cases who had EVD-compatible symptoms and a fever that did not reach the threshold just yet. Indeed, we later witnessed that even the secondary EVD case did not get high-grade fever until several hours after admission to the emergency department [11].

Following the protocol actually delayed the diagnosis of the secondary EVD case from the onset of mild symptoms of malaise and low-grade fever on 30 September until 6 October because there was no reported history of personal protective equipment failure and the presentation of EVD was unusual, i.e.

Table 3

Presence of Ebola virus disease symptoms in the clinical cases consulted with the Department of Public Health Alerts. Community of Madrid, Spain, 1 April–2 December 2014 (n = 215)

Symptoms	n	%	% (cases with symptoms)	n (cases with both clinical and epidemiological case criteria)	% (cases with both clinical and epidemiological criteria)
No	41	19	NA	0	0
Yes[a]	158	73	100	11	100
Fever	124	58	78	11	100
Fatigue	47	22	30	6	55
Headache	45	21	28	6	55
Vomiting	34	16	22	2	18
Diarrhoea	31	14	20	3	27
Myalgia	30	14	19	4	36
Sore throat	27	13	17	2	18
Arthralgia	12	6	8	0	0
Haemorrhagic symptoms	2	1	1	0	0
Unknown	10	5	NA	0	0
Not applicable	6	3	NA	0	0
Total	**215**	**100**	**NA**	**11**	**100**

NA: not applicable.

[a] Cases may have presented with more than one symptom.

Table 4

Recent travel history in relation with consultations on possible Ebola virus disease, Department of Public Health Alerts, Community of Madrid, Spain, 1 April–2 December 2014 (n = 96)

Country	n (%)
Ebola-virus affected countries	
Nigeria (Lagos)[a]	19 (20)
Guinea	6 (6)
Liberia	1 (1)
Mali[a]	2 (2)
Sierra Leone	4 (4)
Ebola-virus affected country but not in the affected provinces	
Democratic Republic of Congo[a]	5 (5)
Nigeria[a]	4 (4)
Countries not affected by the Ebola virus outbreak	
Equatorial Guinea	16 (17)
Mali[b]	6 (6)
Senegal[c]	6 (6)
Morrocco	4 (4)
Tanzania	3 (3)
Côte d'Ivoire	2 (2)
Gambia	2 (2)
Ghana	2 (2)
Other African countries[d]	6 (6)
Europe[e]	3 (3)
The Americas[f]	2 (2)
East Mediterranean[g]	1 (1)
Asia[h]	1 (1)
Unknown	1 (1)
Total	**96 (100)**

[a] Visited during the period of the outbreak (Nigeria: 23 July–20 October 2014, Democratic Republic of Congo: 11 August–20 November 2014, Mali: 23 October 2014–18 January 2015).

[b] Visited when not affected by Ebola virus transmission.

[c] Senegal was never included in the list of affected countries in the Spanish Ebola virus disease protocol.

[d] Angola, Cameroon, Ethiopia, Somalia, Togo and Zambia: 1 inquiry each.

[e] Turkey (n = 2), the Netherlands (n = 1).

[f] Cuba (n = 1), Peru (n = 1).

[g] Saudi Arabia.

[h] China.

paucisymptomatic without clinical signs described in the EVD protocols valid at that time and body temperature below the established threshold [14]. The original national and international protocols had been based on data obtained in outbreaks in Africa and were not sensitive enough for monitoring healthcare workers in contact with an EVD patient. Our experience motivated the European Centre for Disease Prevention and Control to reassess the EVD risk for Europe [24] and led to the adaptation of the EVD protocols to include recommendations for healthcare worker contact monitoring [15,25,26]. After the diagnosis of the secondary case, the criteria for testing an individual for the presence of Ebola virus were applied more loosely and several healthcare workers were isolated and tested even if they did not fulfil the clinical criteria or if there were doubts about direct contact with any of the EVD cases, just to prevent possible further transmission.

An important part of the workload at the Department during the first days of the outbreak was, besides carrying out the epidemiological investigation and tracing the contacts, dealing with inquiries from private citizens who mostly did not fulfil either the clinical or the epidemiological criteria. Speculations about possible routes of EVD transmission in the media (mainly transmission by air and through fomites) caused a lot of anxiety in the neighbourhood of the secondary EVD case: more than two thirds of the inquiries (40/57) from private citizens were related to the secondary EVD case. Two high-risk and one low-risk contact were identified through these inquiries; the remaining 84 contacts were traced through standard outbreak investigation procedures [12]. Many callers experienced at least one EVD-compatible symptom, most commonly fever, headache and gastrointestinal symptoms. But these symptoms have low specificity and may be stress-induced, and many people who thought they had come into contact with the secondary case suffered these symptoms almost immediately after the news were released

[27], even before the incubation period would have been over. The rest of the inquiries were related mainly to recent travel abroad or contact with foreigners or migrants of African origin.

Our data allowed us to evaluate the communication problems that occurred in an emergency situation. Considerable effort was made to raise the awareness about EVD among clinicians and nurses following the arrival of the first repatriated case, but many did not read the EVD protocol, although it was easily accessible online, had been sent out by email and there was a large banner on the homepage of the public healthcare service intranet. The facts that not even 5% of the persons whose cases were consulted fulfilled strictly both the clinical and the epidemiological criteria and that two thirds of the traveller inquiries were not related to areas affected by EVD indicate that one of the fundamental aspects of crisis management in the future has to be active communication with the healthcare workers to avoid unnecessary case classification errors. On the other hand, we have to keep in mind that physicians are not immune to experiencing fear in the face of EVD, that they may worry about the legal consequences of not detecting EVD in a patient or feel responsible for possibly exposing the rest of the healthcare team, other patients and ultimately even their own family to a severe disease. Therefore, it is natural that they choose to contact an epidemiologist in case of doubt. In addition, we must not forget that many medical consultations in primary care and hospitals were resolved correctly without help from the Department. Our results are very similar to those reported by Karwowski et al. who analysed the inquiries received by the CDC from clinicians and local health departments in the US [20]. In their study, 75% of the concerned cases did not have any history of contact with EVD (vs 75% in our study), 21% had travelled to an Ebola-affected country (vs 19% of the clinical inquiries related to travel to an Ebola-virus affected country in our study), 18% had symptoms consistent with EVD and epidemiological risk factors (vs 20% in our study), and 9% were tested for Ebola virus (vs 7% in our study). It is clear that public health authorities need to reassess their communication strategy, making sure their message is heard where it is needed the most, i.e. in the patient examination rooms.

Our experience illustrates the importance of establishing a rapid response consultation service by telephone that offers fast and qualified answers to any questions that may arise during public health emergencies. Such systems may also help find contacts not detected through the epidemiological investigation, as happened in our case. We hope that sharing our experience may help public health professionals in other countries dimension their activities and resources for managing similar exceptional outbreaks in the future.

The Working group of the Madrid Ebola outbreak investigation team

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Conflict of interest

None declared.

Authors' contributions

M.A. López-Pérez and J. Astray-Mochales designed the study. V. Blaya-Nováková performed a literature search, collected the data, analysed the data, and wrote the first draft of the manuscript. All authors interpreted and discussed the results, edited, and commented on the manuscript draft.

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Research article

Development and deployment of a rapid recombinase polymerase amplification Ebola virus detection assay in Guinea in 2015

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In the absence of a vaccine or specific treatments for Ebola virus disease (EVD), early identification of cases is crucial for the control of EVD epidemics. We evaluated a new extraction kit (SpeedXtract (SE), Qiagen) on sera and swabs in combination with an improved diagnostic reverse transcription recombinase polymerase amplification assay for the detection of Ebola virus (EBOV-RT-RPA). The performance of combined extraction and detection was best for swabs. Sensitivity and specificity of the combined SE and EBOV-RT-RPA were tested in a mobile laboratory consisting of a mobile glovebox and a Diagnostics-in-a-Suitcase powered by a battery and solar panel, deployed to Matoto Conakry, Guinea as part of the reinforced surveillance strategy in April 2015 to reach the goal of zero cases. The EBOV-RT-RPA was evaluated in comparison to two real-time PCR assays. Of 928 post-mortem swabs, 120 tested positive, and the combined SE and EBOV-RT-RPA yielded a sensitivity and specificity of 100% in reference to one real-time RT-PCR assay. Another widely used real-time RT-PCR was much less sensitive than expected. Results were provided very fast within 30 to 60 min, and the field deployment of the mobile laboratory helped improve burial management and community engagement.

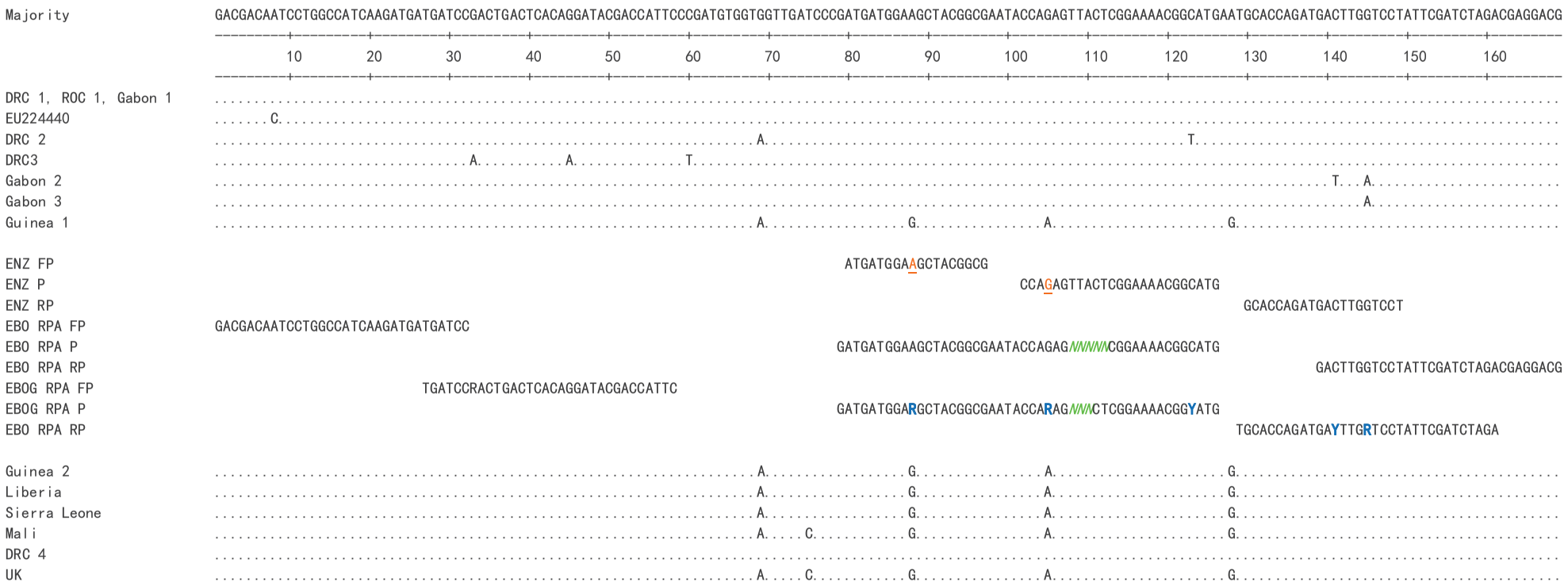
Introduction

As of 11 October 2015, the ongoing Ebola virus disease (EVD) epidemic in West Africa has resulted in more than 28,500 cases and over 11,300 deaths. The early symptoms of EVD (i.e. fever, fatigue, headache, vomiting and diarrhoea) are unspecific and present a challenge for clinical diagnosis [1]. In humans, death occurs generally seven to 10 days after the onset of symptoms. Survivors can be ill for up to 22 days before recovering [2]. Ebola virus (EBOV) infection is mainly diagnosed by various in-house and commercial real-time RT-PCR assays [3], used in up to 38 laboratories implemented at or close to Ebola treatment centres (ETC) in West Africa [4]. Transmission of EVD occurs almost exclusively from human to human by direct contact with body fluids of symptomatic cases. Consequently, the control strategy for EVD epidemics relies on early identification of EBOV-infected patients and corpses for, respectively, isolation and safe burials. It is imperative to trace and follow up contacts and to implement infection control measures.

Therefore, rapid EVD diagnostics impact on outcome of treatment, efficiency of contact tracing and subsequently community engagement, which is central to the successful control of the EVD epidemic. The World Health Organization (WHO) launched a call and consultation for an emergency procedure under its

Figure 1

Alignment of Ebola virus nucleocapsid sequences by country of origin and variant



DRC: Democratic Republic of Congo; ROC: Republic of Congo.

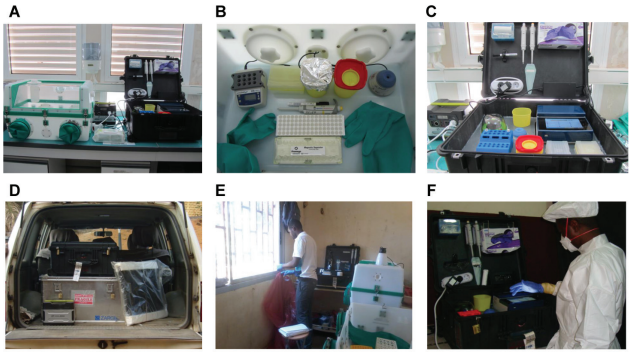
Upper third of the alignment: Sequences from previous and ongoing outbreaks on which the oligonucleotide design was based. GenBank accession numbers: DRC 1 (EBOV Mayinga): NC002549, KC242801, KC242791, AF086833, J04337, AY142960, AF499101, AF272001, KM655246; ROC 1: KM519951; Gabon 1 (EBOV Gabon 94): Y09358, AY058895, EU51640–50, KC242794, KC242798, KC242800; DRC 2: HQ613402–03, KC242784–90; DRC 3 (EBOV Kikwit): KC42796, KC24799, JQ352763, AY354458, AF054908; Gabon 2: EU051639, 2793, KC242795, KC242797; Gabon 3: KC242792; Guinea 1 (EBOV Makona): KJ660346–48.

Centre: Real time-RT-PCR and RT-PRA primers and probes.

Lower third of the alignment: Sequences published since the design. Guinea 2: KP096420–22; Liberia: KM251803, KP178538; Sierra Leone: 99 EBOV genomes [16]; Mali: KP260799–801; DRC 4: KP271018–20; United Kingdom: KP120616, KP184503, KP658432. Degenerated nucleotides are highlighted in bold, mismatches are underlined, NNN represents the gap for the tetrahydrofuran bridge connecting the two sections of the exo probe.

Figure 2

Mobile laboratory for Ebola virus diagnostics



A: Complete mobile laboratory including the solar power pack.

B: The Diagnostics-in-a-Suitcase (DiaS) contains all equipment and reagents to perform up to 100 RT-RPA assays

C: The workspace inside the glovebox contained a heat block mini (VWR International GmbH, Erlangen, Germany), a 96 box each of 100 µl and 1,000 µl sterile filter tips (BRAND, Wertheim, Germany), and two respective automatic micropipettes (Eppendorf AG, Hamburg, Germany), a waste container (Sarstedt, Nuembrecht, Germany), a magnetic separator stand (Promega, Madison, US), a rack for 1.5–2 ml tubes and a marker pen.

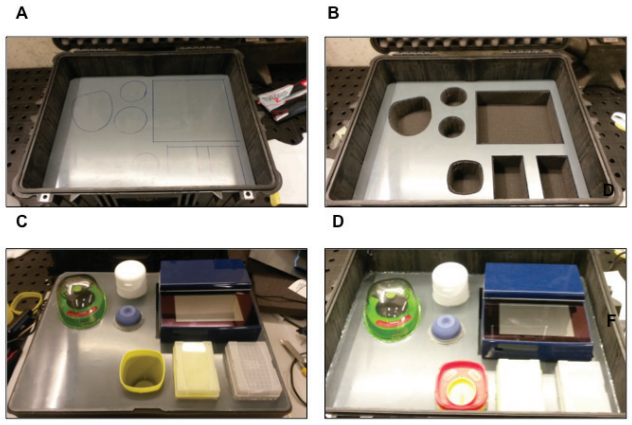
D: Transfer of the mobile laboratory (DiaS, aluminium box containing the glovebox and the PPE, solar panel and power pack).

E: Setup of the glovebox and the DiaS at a hospital in Matoto.

F: Ebola RT-RPA assay in the DiaS.

Figure 3

Assembly of the Diagnostics-in-a-Suitcase



A: A PVC layer was placed on top of the foam filling the bottom of the suitcase.

B: Bespoke insert slots were cut out of the PVC and the foam layer to host the tubescanner, the box for the disinfection wipes, the waste container, the vortex, the minicentrifuge and two boxes of refill pipette tips.

C: Foam, PVC layer and instruments were assembled outside the suitcase. Electricity wires were stowed underneath the foam layer. The equipment was fixed to the PVC layer using hot glue.

D: The setup was placed into the suitcase and the seams were sealed with hot glue.

pre-qualification programme for diagnostic tool assessment [5] to support accelerated development, production and deployment of adapted and rapid Ebola tests. Early in 2015, only three commercial real-time RT-PCR assays (RealStar Filovirus Screen, Altona Diagnostics, Hamburg, Germany; Liferiver Ebola Virus, Shanghai ZJ BioTech Co., Shanghai, China; GeneXpert Ebola virus, Cepheid, Solna, Sweden) and one rapid antigen detection test (ReEBOVTM (Corgenix, Denver, United States (US)) had been approved for emergency use, emphasising the need for such tests. At the time of publication of this article, nine real-time PCR assays for Ebola virus detection have been approved by the WHO.

In this study, we describe the optimisation, evaluation of performance and operational characteristics of a real-time RT-PCR [6] and a rapid RT-recombinase polymerase amplification (RPA) [7] used for diagnosis of suspected Ebola cases, and compare them with the RealStar Filovirus Screen RT-PCR approved for emergency use. In addition, we report the efficient field deployment of the rapid RT-RPA which boosted community engagement for safe and dignified burials.

Methods

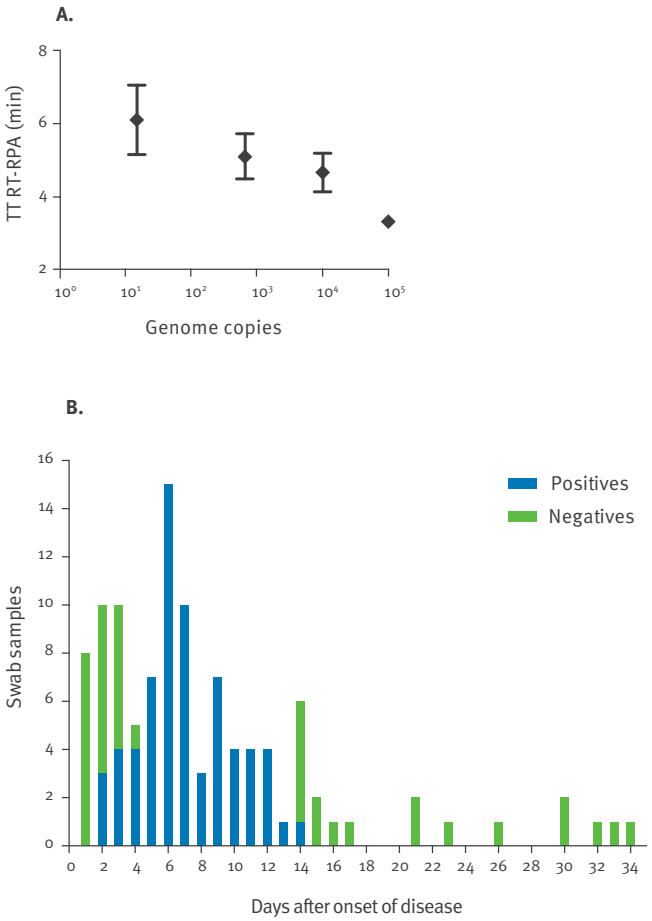
Study design and samples

The study was conducted during the 2014–15 EBOV outbreak in Guinea. On 23 March 2014, the Institute Pasteur de Dakar (IPD), Senegal, upon request of the WHO and the Guinean Ministry of Health deployed a mobile laboratory team to Conakry. An ETC was set up at Donka hospital in Conakry. Serum samples from acute cases and swabs (cheek and tongue) from deceased meeting the WHO definition of a suspected EVD case (see below) were collected in Conakry, Matoto, Télimélé, Coyah and other regions of Guinea between December 2014 and May 2015 and sent to our laboratory for diagnosis. In addition, following an upsurge of EVD cases connected to funeral rites, oral swabs from all deceased were tested at the morgue in Matoto in March and April 2015. During this study, the EBOV RT-RPA was evaluated in parallel to reference methods.

Suspected EVD cases were defined as any person, alive or dead, suffering or having suffered from a sudden onset of high fever and having had contact with a suspected, probable or confirmed case of EVD, or any person with sudden onset of high fever and at least three of the following symptoms: headaches, anorexia/loss of appetite, lethargy, aching muscles or joints,

Figure 4

Sensitivity of the Ebola RT-recombinase polymerase amplification test on (A) inactivated Ebola virus and (B) swab samples, Guinea, December 2014–May 2015 (n=138)



RPA: recombinase polymerase amplification; RT: reverse transcription; TT: threshold time.

A: Plasma samples spiked with inactivated Ebola virus (source: ENIVD) extracted and re-quantified by real-time PCR. Extracts (1.5 × 105, 7.0 × 102, 1.0 × 104, 1.0 × 105 Ebola genome molecules /reaction) were tested in triplicate by RT-RPA. The detection limit was 15 Ebola genome molecules/reaction in a maximum of 8 min.

B: Results from 138 swab samples from deceased suspected EVD cases with symptoms among a cohort of 928 samples from deceased with and without symptoms. Positive and negative results are scored over days after onset of disease.

breathing difficulties, and any person with inexplicable bleeding or any sudden inexplicable death.

RNA extraction and inactivation

Two extraction methods were used. In the first method, viral RNA was extracted from 100 µl serum or swab transport medium using the QIAamp Viral Mini Kit (QC; Qiagen, Hilden, Germany). RNA was eluted in 50 µl Tris-EDTA buffer. The second extraction protocol (SpeedXtract Nucleic Acid Kit (SE), Qiagen, Hilden, Germany) was a reverse extraction method extracting protein debris by way of magnetic beads after an initial 10 min heating step at 95°C. It yielded 200 µl supernatant from 20 µl of serum or oral swab transport fluid diluted 1:2 with molecular grade water.

We added 5 µl of either eluate to the W-PCR (EBOV one-step real-time RT-PCR described in [6]) and the optimised RT-RPA, and 10 µl to the A-PCR (The RealStar Filovirus RT-PCR Kit, Altona-Diagnostics, Hamburg, Germany).

To test for inactivation of EBOV by the new SE kit, SE extract dilutions from 10^{-1} to 10^{-5} were added in triplicate onto 2 × 10^5 VeroE6 cells in a 96-well plate and incubated for five days. The supernatant was passaged three times by transfer to a new well, followed by a 3 h incubation, a wash, and another 48 h incubation step. Finally, cells were washed three times and RNA was extracted in 200 µl Trizol and submitted to an EBOV in-house PCR. For each dilution, three more wells to which the supernatants had been added in the same manner were subjected to an immunofluorescence assay after passage 1 [8]. A not extracted patient serum sample was used as positive control and showed virus growth on VeroE6 cells.

Real-time RT-PCR

The W-PCR was performed on the SmartCycler (Cepheid, Sunnyvale, US) using the RNA Master Hybridisation Probes kit (Roche, Manheim, Germany). A dried 10-fold primer and probe mix containing 100 pmol EBOZ FP and EBOZ RP and 50 pmol EBOZ P (TIB Molbiol, Germany) was used. The A-PCR was used on the SmartCycler according to the manufacturer`s instructions. Positive results above cycle threshold (Ct) 35 were regarded as equivocal and repeated for confirmation [9].

RT-RPA assay

The primers and the exo probe of an existing EBOV RT-RPA assay [7] were redesigned to adopt mismatches of the current EBOV outbreak strain (Figure 1, Table 1) following RPA design guidelines [10].

The RT-RPA was performed using a custom-made EBOV-specific exo RT kit with pellets containing optimised enzyme concentrations similar to the commercial TwistAmp RT exo kit [10,11], and additionally containing primers and probe. Briefly, 5 µl of RNA template and 45 µl of customised rehydration buffer containing magnesium acetate were added to each pellet in a strip of eight tubes delivered in vacuum-sealed pouches. In each strip, tubes 1 to 5 were used to test samples, tube 6 was used as negative extraction control and tubes 7 and 8 for a negative and positive RT-RPA reaction control. The reaction tubes were mixed, centrifuged and then placed into the ESEQuant TS2 (QIAGEN Lake Constance GmbH, Stockach, Germany) for real-time monitoring of fluorescence at 42°C for 15 min, with brief mixing and centrifugation of the reaction tubes after 4 min. The resulting curves were analysed by TS2 Studio Version 1.8.2.0 (QIAGEN Lake Constance GmbH, Stockach, Germany). Increase of fluorescence intensity over time above the mean background signal

Table 1

Primers and probe designed for the updated Zaire ebolavirus RT-recombinase polymerase amplification assay

Name	RPA primers and exo probes
EBOG RPA FP	**TGATCCRACTGACTCACAGGATACGACCATT*C**
EBOG RPA RP	TCTAGATCGAATAGGA**Y**CAA**R**TCATCTGGTGC*A
EBOG RPA P	GATGATGGA**R**GCTACGGCGAATACCA**R**AG-**BTF**-CTCGGAAAACGG**Y**ATG-**Ph**

B: thymidine nucleotide-carrying blackhole quencher 1; F: thymidine nucleotide-carrying fluorescein; FP: forward primer; P: probe; Ph: 3' phosphate to block elongation; RP: reverse primer; T: tetrahydrofuran spacer.

* phosphothioate backbone.

Table 2

Pathogen nucleic acids used for evaluation of the Ebola virus RT-recombinase polymerase amplification assay

Pathogen	Strain/source	Ebola RT-RPA TT (min)	Real-time RT-PCR CT value	Real-time RT-PCR assay
Ebola virus	Zaire strain/BNI	4.7	21.0	ENIVD Ebola standard control and [6]
Ebola virus	GIN/2014/Gueckedou-C05/BNI	5	25.4	
Sudan virus	Sudan Virus Maridi	Negative	22.26	[6]
Bundibugyo virus	Bundibugyo virus	Negative	28.7	In-house assay
Marburg virus	Musoke/BNI	Negative	24.5	[6]
Crimean Congo haemorrhagic fever virus	Kosova Hoti/BNI, Afg09–2990/BNI	Negative	20.3 22.4	RealStar CCHFV RT-PCR Kit ((Altona Diagnostics)
Lassa virus	Josiah/BNI, Lib 1580/121/ BNI	Negative	25.9, 34.7	[16]
Yellow fever virus	Asibi AY640589.1 17D RKI	Negative	20.6, 20.0	[17]
Rift valley fever virus	Strain ZH548	Negative	26.2	[18]
Dengue virus 1–4	VR344 (Thai 1958 strain), VR345 (TH-36 strain), VR216 (H87 strain), VR217 (H241 strain)	Negative	24.2 21.3 23.1 22.7	In-house assay
Zika virus	MR766	Negative	20.86	[19]
Chikungunya virus	A26 Strain	Negative	25.13	In-house assay
Plasmodium falciparum	ND	Negative	15.0	In-house qualitative assay

BNI: Bernhard Nocht Institute; ND: not determined; GIN: Guinea; RKI: Robert Koch Institute; RPA: recombinase polymerase amplification; RT: reverse transcription; TT: threshold time.

Ebola RT-RPA assay identified only *Zaire ebolavirus* but not the nucleic acids of other pathogens.

was analysed by threshold validation (mV/min). Slope validation was used to verify that the increase of fluorescence occurred at a sufficiently high rate, and was verified by first derivative analysis.

The mobile laboratory

The mobile laboratory consisted of a glovebox (Bodo Koennecke, Berlin, Germany), a Diagnostics-in-a-Suitcase (DiaS), and a solar panel and power pack set (Yeti 400 set, GOALZERO, South Bluffdale, US). The disassembled glovebox was kept in a metal box (80 × 60 × 41 cm) with other necessary materials (disinfectant solution, extraction kits, filter tips, racks, vortex, heat block, autoclavable plastic bags and personal protective equipment (PPE). The total weight was 28 kg for the box and 16 kg for the DiaS. Sample inactivation and RNA extraction using the SE kit were done in the glovebox (Figure 2A,B). This allowed handling of hazard group 4 samples. The RT-RPA assay was performed in the DiaS (Figure 2A,C) containing the ESEQuant TS2 device with integrated touchscreen to operate the device and display the results (Qiagen, Lake Constance GmbH, Stockach, Germany). The DiaS was assembled using a trolley case (63 × 50 × 30.2 cm, Peli, Düsseldorf, Germany). The bottom layer of the DiaS contains foam to adsorb shocks during transportation which is covered by a PVC top layer fixed around inserted devices to provide water and chemical resistance (Figure 3, [12]).

Statistical methods

Data were analysed using R (version 3.1.1) [13]. Performance parameters of the test (sensitivity (Se), specificity (Sp), positive (PPV) and negative predictive

Table 3

Evaluation of Ebola virus diagnostic assays, serum and swab samples, Guinea, December 2014–May 2015 (n=1,069)

Extraction method [a]	Sample type	Reference test	Analysed test	Ct range	Analysis values[b]	PPV	NPV	Sensitivity	Specificity	n	Analysed method	Reference method	
												Positive	Negative
QC	Serum	A-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	0.82 [0.72–0.9] 3.2×10^{-9}	0.97 [0.88–1.00] 1.19×10^{-14}	0.97 [0.90–1.00] 4.21×10^{-18}	0.79 [0.60–0.88] 1.04×10^{-6}	141	Positive	68	15
											Negative	2	56
		A-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	0.46 [0.27–0.67] 0.8450	0.97 [0.88–1.00] 1.19×10^{-14}	0.86 [0.57–0.98] 0.0129	0.8 [0.69–0.89] 4.30×10^{-7}	84	Positive	12	14
											Negative	2	56
		W-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	1 [0.93–1.00] 0.178	0.86 [0.75–0.94] 1.57×10^{-8}	0.91 [0.83–0.96] 7.58×10^{-17}	1 [0.93–1.00] 1.78×10^{-15}	141	Positive	83	0
											Negative	8	50
		W-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	1 [0.87–1.00] 2.98×10^{-8}	0.86 [0.75–0.94] 1.57×10^{-8}	0.76 [0.59–0.89] 0.0029	1 [0.93–1.00] 1.78×10^{-15}	84	Positive	26	0
											Negative	8	50
		A-PCR	W-PCR	0–40	Estimate: 95% CI: p value:	0.77 [0.67–0.85] 2.51×10^{-7}	1 [0.93–1.00] 1.78×10^{-15}	1 [0.95–1.00] 1.69×10^{-21}	0.7 [0.58–0.81] 0.0007	141	Positive	70	21
											Negative	0	50
		A-PCR	W-PCR	>30	Estimate: 95% CI: p value:	0.41 [0.25–0.59] 0.392	1 [0.93–1.00] 1.78×10^{-15}	1 [0.77–1.00] 0.0001	0.71 [0.59–0.82] 0.0004	84	Positive	14	20
											Negative	0	50
		W-PCR	A-PCR	0–40	Estimate: 95% CI: p value:	1 [0.95–1.00] 1.69×10^{-21}	0.7 [0.58–0.81] 7.67×10^{-4}	0.77 [0.67–0.85] 2.51×10^{-7}	1 [0.93–1.00] 1.78×10^{-15}	141	Positive	70	0
											Negative	21	50
		W-PCR	A-PCR	>30	Estimate: 95% CI: p value:	1 [0.77–1.00] 0.0001	0.71 [0.59–0.82] 0.0004	0.41 [0.25–0.59] 0.3920	1 [0.93–1.00] 1.78×10^{-15}	84	Positive	14	0
											Negative	20	50
SE	Swab	A-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	0.81 [0.71–0.89] 1.39×10^{-8}	1 [1.00–1.00] 1.20×10^{-240}	1 [0.95–1.00] 1.36×10^{-20}	0.98 [0.97–0.99] 2.86×10^{-212}	881[c]	Positive	67	16
											Negative	0	798
		A-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	0.5 [0.31–0.69] 1	1 [1.00–1.00] 1.20×10^{-240}	1 [0.78–1.00] 6.10×10^{-5}	0.98 [0.97–0.99] 1.12×10^{-213}	828[c]	Positive	15	15
											Negative	0	798
		W-PCR	RT-RPA	0–40	Estimate: 95% CI: p value:	1 [0.96–1.00] 4.14×10^{-25}	1 [0.99–1.00] 3.69×10^{-127}	1 [0.96–1.00] 4.14×10^{-25}	1 [0.99–1.00] 3.69×10^{-127}	928	Positive	120	0
											Negative	0	808
		W-PCR	RT-RPA	>30	Estimate: 95% CI: p value:	1 [0.88–1.00] 3.73×10^{-9}	1 [0.99–1.00] 3.69×10^{-127}	1 [0.88–1.00] 3.73×10^{-9}	1 [0.99–1.00] 3.69×10^{-127}	863[c]	Positive	55	0
											Negative	0	808

CI: confidence interval; CT: cycle threshold; NPV: negative predictive value; QC: QIAamp Viral Mini Kit; RPA: recombinase polymerase amplification; RT: reverse transcription; PPV: positive predictive value; SE: SpeedXtract nucleic acid extraction kit.

[a] Extraction method for RT-RPA. In all cases the reference test was tested with extracts from QC.

[b] Estimated proportions are given in decimals.

[c] This comparison was tested on a smaller subset.

values (NPV) were estimated for each of the assays using real-time RT-PCR assays as reference test. The 95% confidence interval (CI) of performance parameters was calculated based on the exact binomial test. P values are derived from the exact binomial test. The calculated Se and Sp were considered statistically significant for p values < 0.05. We used Fisher's exact test to compare RT-RPA performance parameters in comparison with W-PCR and A-PCR as the reference method at different Ct ranges.

Results

Inactivation

The inactivation of EBOV by the SE extraction procedure was confirmed in VeroE6 cells inoculated with SE extracts which were all negative in IFA. PCR results at passage 4 ranged from Ct 32 to undetectable. Since the IFA was negative, the PCR results were assumed to be due to remnant input RNA but not to actively replicating virus.

Analytical sensitivity and specificity of the RT-RPA assay

W-PCR and RT-RPA detected RNA standards over a range of 5 to 5×10^5 genome copies (GC)/reaction and 50 to 5×10^5 genome copies/reaction, respectively. RT-RPA assays could detect as little as 5 GC/reaction of a molecular RNA standard (data not shown) and 15 GC/reaction in EBOV-spiked human plasma samples (Figure 4A). No cross-detection of important differential diagnostic pathogens or any other filoviruses was observed for the Ebola RT-RPA assay (Table 2).

Performance of RT-PCR and RT-RPA assay using sera

Using a total of 141 sera extracted with QC, RT-RPA and W-PCR performances were assessed using the WHO-approved A-PCR as reference. Against the

Table 4

Significance of the performance analysis results for Ebola virus diagnostic assays, serum samples, Guinea, December 2014–May 2015 (n=141)

	Ct range	RPA/W-PCR	RPA/A-PCR	Fisher's exact test p value
Se	All	0.91	0.97	0.19
Sp	All	1	0.79	3.45×10^{-4}
PPV	All	1	0.82	3.05×10^{-5}
NPV	All	0.86	0.97	0.09
Se	0–20	1	1	1.00
Sp	0–20	1	1	1.00
PPV	0–20	1	1	1.00
NPV	0–20	1	1	1.00
Se	0–30	1	1	1.00
Sp	0–30	1	0.98	1.00
PPV	0–30	1	0.98	1.00
NPV	0–30	1	1	1.00
Se	>30	0.76	0.86	0.70
Sp	>30	1	0.80	3.27×10^{-4}
PPV	>30	1	0.46	1.09×10^{-5}
NPV	>30	0.86	0.97	0.09

NPV: negative predictive value; RPA: recombinase polymerase amplification; RT: reverse transcription; PPV: positive predictive value; Se: Sensitivity; Sp: Specificity.

A-PCR, the RT-RPA yielded a lower PPV (82% vs 100%, $p = 3.05 \times 10^{-5}$), a higher corresponding Se (97% vs 91%, p = 0.19), a higher NPV (97% vs 86%, p = 0.09) and a lower Sp (79% vs 100%, $p = 3.45 \times 10^{-4}$) than against the W-PCR (Table 3 rows 1 and 3, Table 4). The tendency of the results was even more pronounced in the subset of 84 samples with low viraemia (Ct values > 30, Table 3 rows 2 and 4). The difference between the PCR assays was analysed and revealed a reduced Se (77%) for the A-PCR compared with the W-PCR (Table 3 rows 7–8).

Samples determined as positive by the W-PCR but negative by the RT-RPA were also negative in the A-PCR, which missed some additional samples. There was no case of a negative RT-RPA result being positive in the A-PCR (Table 5).

Performance of RT-PCR and RT-RPA assay using swabs

In a preliminary test of RT-RPA efficiency on SE extracts from 47 swabs from deceased patients, all 47 samples scored positive in the W-PCR and the RT-RPA. Therefore, combined SE extraction and RT-RPA were deployed in the mobile laboratory and altogether 928 post-mortem swab samples (including the 47 preliminary ones) were tested. All 928 samples were also extracted by QC and tested by W-PCR and A-PCR. Overall, 120 samples scored positive both in W-PCR and RT-RPA, and only 67 of a subset of 83 samples scored positive in A-PCR. In reference to QC extraction and W-PCR, SE extraction

Table 5

Concordance of results from Ebola virus diagnostic assays, serum and swab samples, Guinea, December 2014–May 2015 (n=928)

Sera	W-PCR	RT-RPA	A-PCR
Positive	91	83	70
Negative	50	58	71
Total	141	141	141
Swabs	**W-PCR**	**RT-RPA**	**A-PCR**
Positive	83	83	67
Negative	798	798	814
Total	881	881	881

Forty-seven additional swab samples were only tested by W-PCR and RT-RPA. In n = 928 samples, these two assays were absolutely concordant.

and RT-RPA yielded a Se and Sp of 100% each (PPV: 100%; NPV: 100%). Since the results of W-PCR and RT-RPA were concordant, the significance of the results was not calculated (Table 5).

The prevalence of positives as tested by W-PCR and RT-RPA in the 928 swabs was 12.9%. Of the 928 post-mortem samples tested, 790 were from suspected cases for whom no signs of disease were recorded and 138 from suspected cases for whom information on symptoms and onset of disease ranging from 1 to 35 days before death were available. Of the 120 positive cases, 53 belonged to the group without recorded symptoms and 67 belonged to the group with symptoms. Positive results were most frequent around day 6 after disease onset and no positive results were obtained later than 14 days after onset of disease (Figure 4B).

Deployment of the mobile laboratory to the local hospital in Guinea

The mobile laboratory was easy to transport to the point of need (Figure 2D-F). The setup of the mobile laboratory including the assembly of the glovebox and donning the PPE took ca 30 min. The SE step was performed in the glovebox for up to 10 samples in 30 min, while the RT-RPA needed 20 min including pipetting steps and mixing. We were able to power the mobile laboratory (peak energy need: 173 W) with the solar battery for up to 16 hours. Before moving to another spot, the glovebox and DiaS were disinfected with 2% bleach or 0.5% incidine. Altogether, setup, operation and disassembly of the unit was easy to perform in a timely manner.

Four Guinean biologists were equipped with and trained in the use of the mobile laboratory at the IPD in January 2015 in a five-day course. After a pilot phase in Guinea, the mobile laboratories were deployed in the Matoto district of Conakry to support testing of swabs from dead suspected cases, which was introduced to

improve community engagement in the EBOV response as well as community surveillance.

Discussion

In this study, we evaluated the analytical and clinical performance of an updated EBOV RT-RPA compared with reference real-time RT-PCR assays. The isothermal RT-RPA assay, which allows real-time detection of amplification from RNA samples using primers and a fluorescent restriction probe within 3 to 15 min [10]. We improved this assay by adaptating the primers to the new sequences of the EBOV strain circulating in West Africa and incorporating them into dried RT-RPA pellets.

In sera extracted by QC, the RT-RPA scored a Se of 91% and Sp of 100% in reference to the W-PCR (Se: 97% and Sp: 79% in reference to the A-PCR), which means it would miss out some weak positives while identifying all true negatives correctly. Results from SE extracted sera were similar (data not shown). Taking swabs is less invasive than taking serum, which makes it more acceptable to populations, but is also safer and easier for sampling and testing. Since SE extraction does not require the use of a centrifuge, we tried to combine the RT-RPA with SE extraction of swabs to simplify our mobile laboratory procedure.

During the analysis of the results, we noted that the widely used A-PCR was less sensitive than the W-PCR. This lower Se was also described by other teams in Guinea and Sierra Leone [14,15]. A rapid detection test (ReEBOAg, Corgenix, Denver, US) was recently scored against the A-PCR with a Se of 91.8% and Sp of 84.6% and approved by the WHO for emergency use. Another recently described rapid detection test also scored a Se of 100% and a Sp of 96.6% against the A-PCR and was rated as a rule-out screening test by the authors because it would include all positives but would miss out on excluding all true negatives, therefore requiring a confirmatory test [15]. Our data confirm their interpretation that the performance of these tests was underestimated when using the A-PCR as reference test.

Our data show that the combination of SE and RT-RPA is superior to the above rule-out tests as all true positive and negative post-mortem oral swabs are detected. Our previous work has shown that magnetic bead extraction is preferable to centrifuge-based extraction under field conditions as it obviates the need for a high-speed centrifuge (unpublished data). We therefore tested the novel magnetic bead-based SE extraction with its 15 min protocol. The materials for both SE extraction and RT-RPA are stable at ambient temperature (30–35 °C) for up to three months and this cold chain-independent combination proved to be well suited for field diagnostics. It scored very satisfactory results in swab extracts (Table 3, rows 13–14), indicating that the RT-RPA does not need a confirmatory test and can be used on site to correctly include positives and exclude negatives.

The prevalence of EBOV in the 928 swabs tested was 12.9%. The day of death after onset of disease peaked at day 6 (range: 2–14 days) in the group of 67 swab-positive deceased for whom disease symptoms were recorded. For the ongoing EBOV outbreak in West Africa, the mean day of symptom onset is 11 days after infection and sera should ideally be collected during the acute phase of illness, within the first 10 days of the disease [2]. We show here that the same is true for swabs, which could simplify diagnostics tremendously. In 53 positive cases, symptoms were not recorded, which was mainly due to a lack of information in the records of the Safe and Dignified Burial teams that did the sampling.

When new EVD foci erupted in previously not affected western parts of Conakry in April 2015, the mobile laboratory was deployed to Matoto to support teams in charge of safe and dignified burials. Since it had been decided that all deceased should be tested, these teams collected swab samples from deceased of five neighbourhoods of Conakry (Matoto, Ratoma, Dixinn, Matam and Kaloum) and up to 50 samples had to be tested per day. The emergency response results were provided every 30 to 60 min to the field investigators and physicians. The rapidity and mobility of the RT-RPA method in the DiaS, in comparison with the average 3 to 4 h turnover with regular real-time RT-PCR, was appreciated by burial teams, health authorities, response teams and communities, as it allowed rapid clearance for normal burials deceased persons who were confirmed negative. The results also encourage the use of swabs from patients at ETCs. In that context, it would still be necessary to determine if swab samples can replace sera samples.

The deployment demonstrated that the mobile laboratory using glovebox, DiaS, SE and RT-RPA is a very good solution for decentralised biosafe diagnosis of EBOV, resulting in direct impact on community engagement for disease control. Moreover, this small mobile laboratory run by local teams is a sustainable contribution to future outbreak control.

Addendum (28 January 2016)

The World Health Organisation (WHO)-approved RealStar Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) has been widely used on Smart Cyclers throughout the Ebola outbreak in West Africa, although the manufacturer does not list this device in their instructions for use. The company published a note on its website on 10 July 2015, commenting the reported lack of sensitivity of this kit when used on a Smart Cycler. According to our findings, we consider that the reported lack of sensitivity is related to the restriction of using the kit for specific PCR cyclers only, as also found by other groups [20] previously.

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Conflict of interest

Oliver Nentwich and Olaf Piepenburg are employees of TwistDx Ltd, a wholly owned subsidiary of Alere Inc. The RPA technology is subject to background IP protection and is owned by Alere.

Authors' contributions

AAS, MW, AAEW, PP, OuF designed the study. AAS, MW, AAEW, PP wrote the manuscript. OuF, OsF, BS, AM, DK, AAS, NM collected the data. ON, OP developed and provided primer-in pellets, GF, SK, NF, MKK, AAD, LK, MN organize and support the field deployment. HK, AM performed the inactivation study. All authors contributed to analyse the data and reviewed the manuscript.

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Preparing for imported Ebola cases in Israel, 2014 to 2015

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During the current outbreak of Ebola virus disease (EVD) in West Africa, preventing exportation of the disease posed many challenges for economically more developed countries. In Israel, although the risk of importing single cases was assumed to be low, the implications of local transmission were great. This article describes the EVD preparedness plan of the Israeli Ministry of Health. Key elements were a sensitive case definition, designation of a single treatment centre for suspected and confirmed cases, construction of a mobile unit using customised negative-pressure tents and a vigorous national training programme. There were no patients with EVD in Israel, but a few suspected cases were assessed. The Israeli plan may provide a template for emergency infectious disease response in other geographically small countries.

Introduction

The recent epidemic of Ebola virus disease (EVD), beginning in late 2013 in West Africa, was the largest ever reported, with a case count of over 28,500 and more than 11,000 deaths until 28 October 2015 [1]. The vast majority of cases were concentrated in three West African countries, with only 22 cases exported to or presented at eight European countries and the United States (US) [2-8]. However, the failure of local medical systems to control the outbreak and the presence of foreign medical teams in the affected countries raised concerns about exportations to other countries among the public, healthcare professionals and government authorities. These fears increased following reports of three cases of nosocomial transmission in the US and in Spain [4,9]. Many efforts and resources were invested worldwide to prepare for EVD exportation.

This report describes the national preparedness plan for EVD in Israel, with its unique characteristics and solutions.

Response planning

The Israeli preparedness plan for EVD was developed and executed by the Israeli Ministry of Health (MOH), with advice from the Epidemic Management Team (EMT), a multi-disciplinary task force supporting decision making about biothreats comprising members from every relevant health profession and other organisations. The EMT adheres to the international guidelines of the World Health Organization (WHO), the Centers for Disease Control and Prevention (CDC) in the US and the European Centre for Disease Prevention and Control (ECDC). Published lessons, data and cases from West Africa were tracked, and expert teams were appointed to advise the EMT on specific problems. The MOH rapidly issued regulations granting its Director General special powers in dealing with potential EVD cases, including the right to enforce examination and to monitor, isolate, quarantine and treat suspected patients and their contacts.

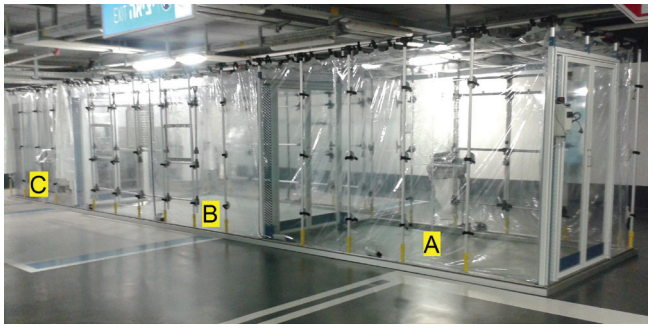
Media communication was coordinated proactively by the MOH headquarters. Overall, the media and the public were accepting of the government measures.

Risk assessment

An assessment of the potential for international spread of EVD through routine travel from the three affected countries estimated an average of 2.8 exported cases globally per month [10]. Given that there was little direct communication and no direct flight routes between Israel and the countries affected by EVD, the probability of cases originating from returning citizens or travelling foreigners was assumed to be very low. A higher risk was anticipated were the epidemic to spread to other African countries that have closer ties with Israel. The possibility of disease in an Israeli healthcare worker (HCW) returning from an Ebola-affected area was considered, but no restrictions were

Figure 1

The mobile IsoArk, an isolation unit used in the Ebola Treatment Center, Israel, 2015 A



The unit contains three separate chambers with increasing negative pressure and unidirectional airflow.

A. Wide anteroom for the passage of staff and equipment and doffing of personal protective equipment.

B. Main treatment area, size: 5.3 × 3.2 m, equipped as an intensive care unit.

C. Shower, toilet and waste treatment unit

Figure 2

Bathroom and toilet facilities inside the IsoArk isolation unit

All liquid waste is actively pumped into a decontamination system located outside the tent.

placed on Israeli volunteers. Very few Israeli volunteer HCWs reported treating of EVD patients directly, and an estimated 50 Israeli aid workers participated in logistical and psychosocial support missions without direct contact with patients.

Even in the three affected countries, with poor hygienic conditions and healthcare infrastructure, the reported level of contagiousness of EVD was not high, with mean basic reproduction rates (Ro) ranging from 1.71 to 2.02 in Guinea, Sierra Leone and Liberia [11]. Therefore, a very limited number of secondary cases was expected, especially if stringent infection control protocols and active surveillance of incoming travellers were implemented. With these considerations, the MOH directive was designed to prepare for up to four patients with EVD simultaneously (one or two imported patients and up to two secondary cases).

Case definition

Case definitions of suspected EVD developed over time and varied by country [12,13]. Owing to the small number of potential cases expected in Israel, a relatively sensitive suspected case definition was selected: any person who presented with a fever of 38 °C or more and had visited a country with widespread EVD transmission within 21 days before symptom onset [14]. Confirmed cases were defined by a positive PCR test for Ebola virus. Suspected cases were designated as such only on approval of the public health services (PHS), mandating early national involvement in every single case. Entry screening was implemented, with 21-day follow-up for all travellers arriving from endemic countries. The MOH guidelines called for the exclusion of other common causes of fever in returning travellers, especially life-threatening diseases such as malaria. This was to be done only in the designated Ebola centre.

Infection control

The principle route of EVD transmission is contact with contaminated body fluids, primarily vomit, faeces and blood [15,16]. Epidemiological data do not support airborne transmission, and secondary cases have been described almost exclusively among household contacts and persons in direct contact with patients. Nevertheless, it was argued by some that airborne transmission may occur [17], and the WHO and the CDC recommend that HCW apply airborne precautions during aerosol-generating procedures such as intubation and suctioning, and in events of spillage of contaminated excretions [15,18]. A small number of animal studies have suggested airborne pig-to-primate and primate-to-primate transmission [19,20]. Furthermore, owing to their stability in aerosols, filoviruses are considered a potential category A biological weapon [21-23]. Therefore, the MOH incorporated the universal recommendations for airborne precautions, including placement of the patient in negative-pressure isolation rooms, and use of N95 (FFP3) respirators by caregivers, simplifying protection recommendations and avoiding accidental airborne exposures.

Personal protective equipment (PPE) for caregivers was specified and purchased centrally by the MOH and distributed to all acute care hospitals in the country and other relevant caregivers. The overall cost for PPE and additional supplies for infection control was estimated

as EUR 1 million. The standard PPE kit for all HCWs includes hospital scrubs, rubber boots, water-resistant coveralls (EN 14126 biological protection standard), water-resistant shoe covers, two pairs of nitrile gloves, an N95 respirator, a hood to cover exposed areas of the head and a face shield. In addition, the MOH recommended the use of a water-impermeable coverall under conditions of heavy exposure to liquid spillage, such as bathing the patient or cleaning the room. The ministry also prescribed a specific universal donning and doffing procedure [14]. Safe doffing was ensured by co-worker supervision and rinsing of gloved hands with bleach at each stage.

Although internal medicine departments in Israeli hospitals have isolation rooms, these were considered inappropriate for patients with EVD because they did not allow for optimal spatial separation from the surrounding rooms, precluding care of non-EVD patients in the same department. Furthermore, although Israeli public knowledge about EVD was reported to be quite good [24], hospital administrators were very reluctant to designate these rooms for EVD patients for fear of stigmatising entire wards or even buildings. Such perceptions, although scientifically unsubstantiated, are not uncommon in infectious disease outbreaks [25]. Therefore, a portable, modular, free-standing isolation unit was constructed using customised negative-pressure tents (IsoArk, Beth-El Industries Ltd, Zikhron Yaakov, Israel). The tent, originally designed in 2003 for the isolation of patients infected with the airborne severe acute respiratory syndrome (SARS) virus, contains an anteroom for safe passage of staff and equipment and a room used as an intensive care unit. In order to adapt the unit to EVD patients, a 'wet' room with running water and a toilet was added. Airflow is unidirectional, and an external decontamination system inactivates drained liquid waste (Figure 1 and Figure 2).

Allocation of roles within the Israeli health system

The preparedness plan involved all components of the Israeli health system. Because of the limited time available to train staff and ensure the highest infection control standards, the MOH designated a single hospital to serve as the national Ebola treatment centre (ETC). Rambam Medical Center in Haifa was chosen because of its excellent infection control practices and a large emergency infrastructure, which supported the construction of isolation facilities outside its main campus. An underground complex that is intended to serve as an all-hazards emergency hospital was selected as the optimal site, to be staffed by volunteers from various hospital departments. The ETC was eventually housed in free-standing IsoArk units, containing patient rooms and an on-site laboratory. Provisions were made to support intensive care, including the spacious patient rooms (5.3 × 3.2 m), necessary equipment for respiratory and haemodynamic support and intensive care staff. Renal support could theoretically be provided by plasmapheresis. Laboratory capabilities included blood count, basic chemistry, blood gas analysis and coagulation tests. Imaging was based on a portable X-ray unit inserted through a dedicated plastic sleeve near the patient's bed and a digital cassette wrapped in plastic bags. Separated sections were allocated for donning and doffing and provisions were made for autoclaving of all solid waste. Patients thus had access to tertiary level care without exposing general hospital facilities to potential Ebola virus contamination.

All other hospital emergency departments and community clinics were instructed to provide only life-saving care for suspected cases while adhering to strict isolation and personal protection practices as long as the patient was under their care. They were to immediately report the case to the district public health officer, followed by transfer to the ETC, without performing any laboratory or imaging work-up [14]. This delay in patient care was considered acceptable given Israel's size: ground transport to the ETC could be expected to take less than four hours. The different hospital roles were reflected in the differential distribution of PPE, intended to last only for a few hours in all hospitals except the ETC, which was equipped for a lengthy hospitalisation.

Magen David Adom (MDA) is the main emergency transport service in Israel, with extensive experience in all types of emergency evacuations. MDA designated and trained specific teams for each district as the sole personnel authorised to transport patients with suspected or confirmed EVD. The teams used a different PPE from other caregivers, with advanced chemical and biological protection including powered air purifying respirators (PAPR), because they were already familiar with this equipment. They were further equipped with negative-pressure patient transport units.

Patient samples were tested for EVD by PCR at a national-level high-safety laboratory. A detailed protocol for the safe collection, packaging and transport of specimens was issued [14].

Travellers from affected countries arriving in Israel were identified at air and sea borders both voluntarily, using informative posters and leaflets, and by border control officers reviewing passport logs. Travellers' temperatures were taken with a non-touch thermometer by personnel wearing gloves and a face shield. Symptomatic travellers were to be interviewed by airport medical staff and transported to the ETC. No symptomatic travellers have so far been identified by these controls. District public health offices were responsible for conducting follow-up of asymptomatic travellers twice a day for 21 days, and for epidemiological investigation of symptomatic cases and contact tracing, in a similar way as described by CDC [26]. Public health officers were instructed to minimise physical contact with suspected cases with the help of distance-enabling technologies (intercoms, video cameras, etc.).

The PHS were responsible for executing the preparedness plan on the national level, by distributing updated guideline to the medical community, border control, other emergency response organisations and the general population, and by enlisting professional advice and assistance from the EMT and experts around the country as needed [14].

Assessment of suspected cases in Israel

Between October 2014 and February 2015, 80 asymptomatic travellers were identified and followed; none of them developed symptoms. However, some travellers arriving from West Africa circumvented border screening by avoiding public health staff and presenting themselves using a passport with no documentation of presence in the affected countries. Three febrile patients were assessed for suspected EVD, one before and two after the national guidelines became available. The latter two had evaded border identification and hence were not actively followed by PHS before presenting to the hospital. All three tested negative for Ebola virus and were diagnosed with other infectious diseases.

Healthcare worker training

Maintaining HCW safety was a cornerstone of the preparedness plan. Events in Africa, the US and Spain indicated that even excellent PPE is worthless without effective donning and doffing techniques and patient care practices. Therefore, the MOH instigated a crash programme to familiarise HCWs with PPE and infection control principles. These measures were aimed at building up confidence among HCWs, alleviating stress and easing any reluctance to participate in EVD patient care. The main topics covered were risk assessment for occupational EVD exposure, stringent contact precautions, proper PPE donning and doffing and vital steps in dealing with a patient with suspected EVD. Ninety briefings for hospitals, law enforcement and border control personnel were held by MOH staff, aided by military personnel qualified in PPE instruction. Briefings were followed by hands-on training. Proficiency was tested in 30 surprise drills, conducted at least once in every acute care hospital. These short drills simulated the admission of a suspected patient to the emergency department, followed by rapid assessment and transportation to the ETC. MOH staff evaluated adherence to the national guidelines and infection control practices. Media representatives were actively invited to participate in order to emphasise publicly the preparation efforts.

Discussion

Epidemics require complex responses. Although there are many similarities in the response plans for different diseases, no one generic plan can answer all eventualities. The recent EVD epidemic was characterised by a high fatality rate, lack of effective treatment or vaccine, and unclear mode of transmission. All of these led to anxiety among the general public and the healthcare community. Accordingly, the level of preparation and precautions taken in countries outside Africa was unprecedented.

Israeli health authorities constructed a comprehensive preparedness programme in anticipation of EVD importation from Africa. Although the risk was perceived to be low, up to four patients including limited local transmission, the implications were profound. Several elements of the Israeli response were unique to the EVD epidemic: follow-up of inbound travellers, designation of specific transport teams and a single medical centre and the stringent application of PPE. Experience with a handful of suspected cases, later found to be negative, validated this exacting approach. Similar actions in the US and the United Kingdom later reinforced the designation of regional ETCs [27,28].

International infection control recommendations call for extreme precautions to prevent contact transmission of EVD and for some airborne precautions during aerosol-generating procedures. According to the WHO, solid waste needs to be burned or buried and liquid waste drained into the general sewage [15]. Nevertheless, most countries in the developed world implemented even stricter procedures during the EVD epidemic, such as use of PAPR, isolation in negative-pressure rooms, and chemical or physical inactivation of any waste. Some countries relied on existing high-level isolation units (HLIU) [29-32], but others, including Israel, had no such capability [33]. Much of the isolation infrastructure in Israel was developed as part of the response to SARS, smallpox and other airborne diseases and was not optimally suited to the typical EVD patient who produces large amounts of liquid and solid waste. In addition, hospital administrators were reluctant to have patients with EVD on the same ward as other patients. As construction of a HLIU was impossible in the existing time frame, the MOH decided to construct a free-standing negative-pressure isolation unit using customised tents, combined with appropriate sanitary and waste disposal facilities. In this manner, patients could be maintained in a highly secluded area within reach of a hospital yet far from hospital personnel and the public. This approach proved practical, easy to develop and relatively inexpensive compared with HLIU construction. The MOH is currently contemplating the construction of a HLIU in Israel; until then, the ETC serves as a practical solution for the treatment of future patients with highly contagious and hazardous diseases.

Even with traveller surveillance and guidance, unexpected cases of EVD might present at any emergency department. Thus, the MOH found it necessary to prepare every hospital in the country. Prompted by findings from Europe that only 16% of hospitals that were not intended to admit EVD patients, and 46% of all admitting hospitals had undergone preparatory exercises [29], the MOH included a vigorous educational campaign in its plan. The programme included educational sessions, combined with hands-on practice and

surprise drills, and was successfully delivered to all acute-care hospitals in Israel within a few weeks.

Conclusion

In summary, we describe the preparedness programme of the Israeli MOH in response to the threat of EVD importation during the 2014–15 epidemic in West Africa. Although no patient was diagnosed with EVD in Israel, training and treatment of suspected patients showed that the plan was effective and manageable. A national protocol that relies on one specialised treatment unit, together with a moderately low-cost and rapidly constructed isolation facility, enabled a high level of care under significant economic constraints. Israel's programme may provide a template for emergency infectious disease response in other geographically small countries.

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Conflict of interest

None declared.

Authors' Contributions

All the authors had a prominent role in the construction of the Israeli preparedness programme. TBN drafted the article. All authors participated in manuscript writing and revision. All authors read and approved the final manuscript.

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Public health challenges and legacies of Japan's response to the Ebola virus disease outbreak in West Africa 2014 to 2015

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The largest outbreak of Ebola virus disease occurred in West Africa in 2014 and resulted in unprecedented transmission even in distant countries. In Japan, only nine individuals were screened for Ebola and there was no confirmed case. However, the government promoted the reinforcement of response measures and interagency collaboration, with training and simulation exercises conducted country-wide. The legacies included: publication of a communication policy on case disclosure, a protocol for collaboration between public health and other agencies, and establishing an expert committee to assemble the limited available expertise. There were challenges in taking proportionate and flexible measures in the management of people identified to be at high risk at entry points to Japan, in the decentralised medical response strategy, and in the medical countermeasures preparedness. The Ebola outbreak in West Africa provided a crucial opportunity to reveal the challenges and improve the preparedness for rare but high impact emerging diseases that are prone to be neglected. Efforts to uphold the lessons learnt and maintain public health preparedness should help prepare for future emerging diseases, including bioterrorist acts and pandemics.

Introduction

The outbreak of Ebola virus disease (EVD) in West Africa in 2014 was the largest outbreak in history, and the World Health Organization (WHO) declared a Public Health Emergency of International Concern (PHEIC) on 8 August 2014 [1]. Importation and transmission to non-endemic countries and evacuation and repatriation outside Africa [2,3] forced public health authorities to prepare for EVD even in countries far away from the outbreak.

Japan has not experienced a case of viral haemorrhagic fever (VHF) since 1987, when a case of Lassa fever was imported from Sierra Leone [4]. The Act on Prevention of Infectious Diseases and Medical Care for Patients with Infections in Japan (the Act), which came into effect in 1999, categorises EVD as a Category 1 infectious disease (Category 1 disease), along with other viral haemorrhagic fevers, plague, and smallpox [5]. The Ministry of Health, Labour and Welfare (MHLW) and the local prefectures have designated specified infectious disease hospitals (Specified hospitals) and Class 1 infectious disease hospitals (Class 1 hospitals), respectively, to provide treatment for Category 1 diseases in an isolated biosafety ward at public expense (Table 1). Category 1 diseases are also quarantine diseases in the Quarantine Act.

Although the number of travellers with citizenship of the three EVD endemic countries was limited to approximately 300 to 500 per year [6] and no direct flight is operated to and from those countries, the Government of Japan (GOJ) started to reinforce the border controls and domestic response capacity from August 2014 onwards. As of the end of September 2015, at least 20 healthcare workers from Japan have been deployed through the WHO Global Outbreak Alert and Response Network (GOARN) network. Only nine people were screened for EVD in Japan up to September 2015 (Table 2) [7]. All were negative and no case was reported among Japanese citizens overseas either. Still a government-wide response system promoted reinforcement of response measures and interagency collaboration.

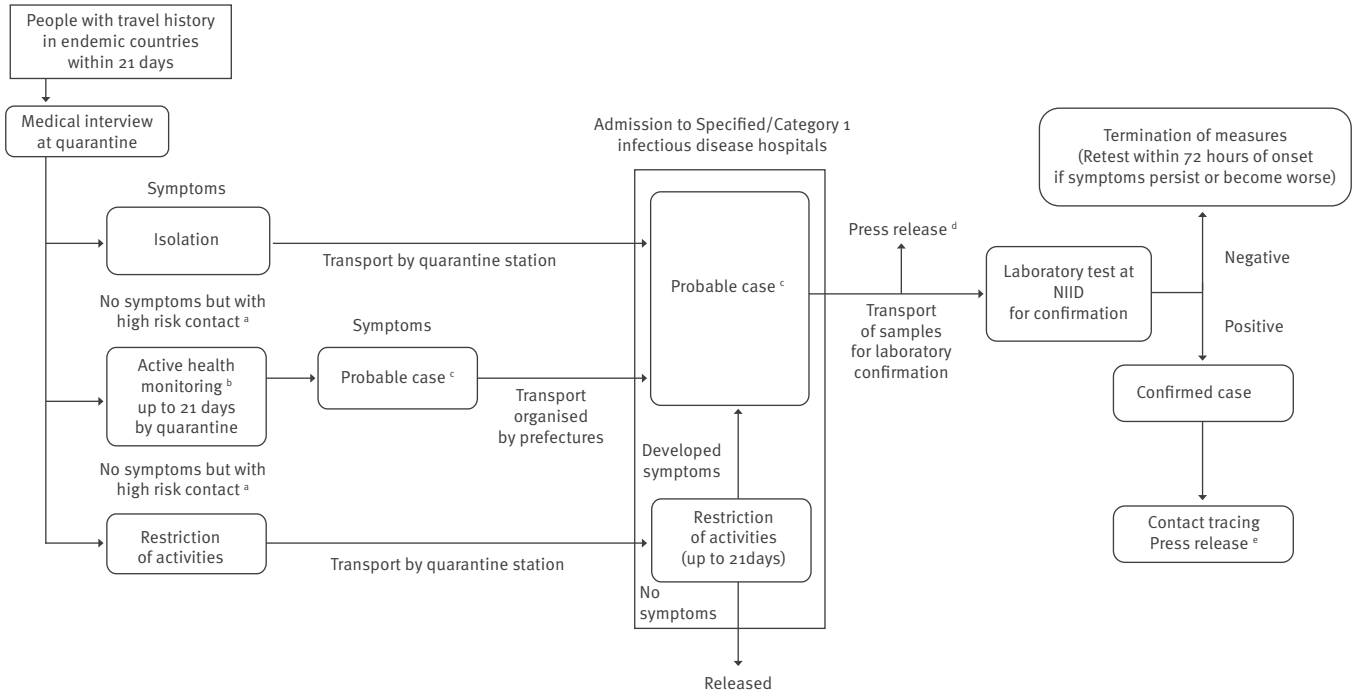
Here, the author reviews the public health and medical preparedness and response in Japan to the 2014 EVD outbreak in West Africa, and discusses the legacies and challenges for the preparedness and response to emerging diseases.

Border measures and management of people travelling to Japan from endemic countries

The GOJ reinforced border measures for containment of EVD from August 2014 by raising awareness among

Figure

Case management protocol for people with a history of travel to Ebola virus disease endemic countries within the previous 21 days, Japan, 21 November 2014–17 September 2015 [modified from reference 9]



EVD: Ebola virus disease; NIID: National Institute for Infectious Diseases.

[a] High-risk contact i.e. direct contact with a virus through mucosa or wounds or by needle-stick injury .

[b] Individuals with at high risk of contact with a virus may be asked to stay at home.

[c] A probable case was defined on 24 October 2014, as i) travel history to endemic countries within 21 days and fever OR ii) contact history to body fluids of patients and feeling of warmth. In order to emphasise the contact history, the definition was modified on 18 September 2015 as i) fever >38 °C or clinical symptoms of EVD AND 2) history of contact with body fluids through EVD patients OR history of contact with bats and primates in endemic countries within 21 days.

[d] A press release will be issued when a possible case is identified and blood samples are sent from the hospital to the NIID for diagnosis. The following patient data will be disclosed: age group, sex, prefecture of residence or nationality, travel history, contact history in the endemic country, symptoms, diagnosis of other infectious diseases, flight information for those developing symptoms at quarantine, prefecture of admitting hospital, day of departure from the endemic country and day of onset of symptoms developed during health monitoring [7].

[e] A press release will be issued when the case is laboratory-confirmed.

travellers entering Japan at quarantine stations from 1 August. Travellers were asked to declare their travel history to endemic countries and to contact local public health centres if they developed symptoms described in several communication channels, such as posters at quarantine stations and in-flight announcements. Starting with 24 October 2014 (at airport) and with 21 November (at seaport), immigration control officer also asked travellers if they had been in or travelled from an endemic country. Those with a history of contact with EVD patients were isolated if they had symptoms, or were placed under health monitoring [7].

The GOJ considered strengthening border measures after several reports of cases in non-endemic countries from September 2014 [2,3], and a large increase in numbers of patients in endemic countries. The case of a patient in Japan who had a history of travel to Liberia but did not attend a quarantine station and visited a hospital without his travel history being noted [8], further convinced the GOJ of the need to strengthen the management of people with a travel history to endemic countries.

On 21 October 2014, the MHLW revised the entry screening policy to define those who had travelled from Guinea, Liberia, or Sierra Leone within 21 days as having a history of contact with Ebola patients [7]. According to the Quarantine Act, these travellers were to be isolated if they had symptoms at quarantine, or to be put under active health monitoring i.e. having to report body temperature and conditions to a quarantine station twice a day for 21 days after the last visit to any of the three countries. The definition of a probable

Table 1

Function and roles of designated infectious disease hospitals under the Infectious Disease Control Act, Japan, as at 10 November 2014

	Type of infectious disease hospital		
	Specified	Class 1	Class 2
Designated by	Minister of Health, Labour and Welfare	Prefectural Governor	Prefectural Governor
Location policy	Several nationally	One in a prefecture	One in a secondary medical care area [e]
Major requirements for the wards	Not specifically documented	Negative pressured private room with toilet and shower Anteroom Dedicated ventilation with HEPA filter Dedicated drainage	Dedicated ward for infectious disease patients with toilet and shower
Diseases	Novel [a], Category 1[b], 2[c]	Category 1[b], 2 [c]	Category 2 [c]
Hospitals (beds)	3 (8) [d]	45 (86) [d] in 38 of 47 prefectures	335 (1,716)

HEPA: high efficiency particulate air.

[a] Novel infectious disease is a category for an emerging severe and highly transmissible disease with unknown aetiology

[b] Category 1 infectious diseases include smallpox, Ebola haemorrhagic fever, Marburg disease, Crimean-Congo haemorrhagic fever, Lassa fever, South American haemorrhagic fever and plague.

[c] Category 2 infectious diseases include acute poliomyelitis, diphtheria, severe acute respiratory syndrome, tuberculosis, and specified avian influenza virus infections (H5N1 and H7N9).

[d] Two hospitals are designated as both Specified and Class 1 infectious disease hospitals.

[e] Approximately one in several municipalities

case which should be hospitalised in a designated hospital, as well as a confirmed case, was modified to emphasise the travel history to endemic countries (Figure). Thus, if people developed symptoms during monitoring, they were to self-isolate, call a quarantine station, and be hospitalised in a designated hospital. The response protocol for local health authorities was revised accordingly on 24 October 2014. The MHLW added measures on 21 November for those who were asymptomatic but had a high risk of EVD, asking them to stay home (Table 3) [9].

Challenges in management of people travelling to Japan from endemic countries

Measures for Category 1 diseases under the Act and the Quarantine Act can be taken not only for a confirmed case, but also for a probable case, which is clinically diagnosed but not laboratory-confirmed. These measures were intended to be implemented as early as possible for those who had a high probability of infection when they developed symptoms. Because the early symptoms of VHFs are non-specific, the definition of a probable case should be applied cautiously to avoid imposed restriction of freedom of movement.

The modified definition of a probable case increased the sensitivity for detecting people at risk of EVD, and may have reassured the media and public when there was an epidemiological uncertainty about transmission mechanisms. There was a concern that those who visited endemic countries may have not recognised the contact to EVD patients. However, a higher sensitivity of a case definition may result in a larger number of people incorrectly classified as cases, for which unnecessary measures are enforced. A case reported on 7 November 2014 during active health monitoring had to be transported in an isolated unit from home to hospital under intense media attention, even though he did not have a contact history with EVD patients, and had been diagnosed with tonsillitis at a local clinic. Measures which are too strict and disproportionate may make people hesitate or reluctant to declare their symptoms voluntarily, which is the basis of modern policies of infectious disease control, and this will result in a negative impact on public health. In fact, the above mentioned case visited a clinic without giving notice to a quarantine station and did not tell the doctor about his travel history to Liberia despite instructions from a quarantine station during active health monitoring [10].

The protocol above had been active until it was revised on 18 September 2015, to emphasise the contact history to a patient or bats for the definition of a probable case. Special measures such as described here should be applied for a limited duration only and be reviewed periodically for appropriateness as the scenario changes to balance public health needs and rights of the individual of free movement.

The legacy in case management was a communication policy on possible EVD cases identified at quarantine and within the country (Figure) [7] to clarify the timing and contents of disclosure. The disclosure policy on a

Table 2

Cases screened for Ebola virus disease, Japan, August 2014–September 2015 (n=9)

Age groups (years)	Sex	Visited country	Nationality	Symptoms	Contact history	Reporting	Diagnosis
20–29 (n=1) 30–39 (n=2) 40–49 (n=4) 50–59 (n=0) 60–69 (n=1) 70–79 (n=1)	Male (n=7) Female (n=2)	Guinea (n=4) Liberia (n=3) Sierra Leone (n=2)	Japan (n=6) Guinea (n=2) Undisclosed (n=1)	Fever (n=9) Body pain (n=2) Chill (n=1) Cough (n=1) Headache (n=1)	None (n=7) Contact to body bag (n=1) Undisclosed (n=1)	During health monitoring (n=6) At quarantine (n=3)	Malaria (n=4) Influenza (n=1) Others (n=4)

Table 3

Classification of contact with confirmed cases of Ebola virus disease or related fluids [modified from reference 24], Japan, as at 21 November 2014

Type of exposure	Appropriately protected	Unprotected or inappropriately protected
Direct contact with a virus to mucosa or wounds or by needle-stick injury	NA	High risk [b]
Contact with body fluids of cases	Low risk [a]	High risk [b]
Those handling specimens of cases	Low risk [a]	High risk [b]
Medical examination, procedure or transportation of cases within 1 meter	Low risk [a]	High risk [b]
Other staff involved in the medical management or transportation of cases, those living with the cases	Low risk [a]	Low risk [a]

NA: not applicable.

[a] Should be under active health monitoring without movement restriction.

[b] Should be under active health monitoring and are asked to stay at home.

case of an emerging disease has always been an issue of concern [11], and the protocol will provide a basis for such an event in the future.

Collaboration of public health sectors with other agencies

The GOJ activated the government-wide crisis response system on 28 October 2014, immediately after the first traveller returning from Liberia to Japan developed fever at quarantine. The first ministerial meeting on the response to EVD was held with the participation of the Prime Minister. After that, the response and measures of relevant ministries and agencies were coordinated at the Intergovernmental Coordination Meeting on EVD measures, chaired by the Deputy Chief Cabinet Secretary for Crisis Management [12].

Progress was made in coordinating public health agencies with the fire department and police to safely transport patients and clinical samples. The Ministry of Internal Affairs and Communications agreed with the MHLW on the arrangements for transportation of confirmed or probable cases of EVD by the local fire departments when requested by local public health centres [13]. The National Police Agency also agreed with the MHLW on the assistance required for emergency transportation of a confirmed or probable case of Class 1 disease and its clinical samples [14]. These documented agreements represent a key legacy for future cooperation among the relevant agencies.

Planning and preparedness should be tested by regular simulation exercises to strengthen the collaboration between the relevant agencies. On 3 November 2014, the MHLW requested local governments to practice the protocol for transporting patients and samples. All 141 local governments and municipalities with public health centres completed the exercises before the end of March 2015. The next challenge is to maintain this collaborative network.

Challenges for domestic medical response capacity for Category 1 diseases

The primary strategy to provide medical care for VHF patients in Japan (population: 127 million as of May 2015 [15]) was to place a designated Class 1 hospital in each prefecture, because the authority for controlling infectious diseases is decentralised to 47 prefectures under the Act. Even though the incidence of Category 1 diseases may be very low, Class 1 hospitals are expected to play a role as core hospital to improve medical care for infectious diseases in the prefecture [16]. The cost of establishing and operating a Class 1 hospital has been subsidised directly by prefectures

and indirectly by the MHLW; however, even 15 years after the Act was enforced, nine prefectures had not yet set up a Class 1 hospital at the declaration of the PHEIC in August 2014; two prefectures did however designate Class 1 hospitals in November 2014 and March 2015 [7]. To improve response capacity, training in the use of personal protective equipment was provided for 328 local public health officials and workshops were provided in 19 of 45 Class 1 hospitals from October 2014 to February 2015. The National Center for Global Health and Medicine, a Specified hospital, prepared a team to assist a Class 1 hospital in case of an emergency [12].

Maintaining high-level biosafety care facilities and trained staff in every prefecture may have been a too idealistic goal for continued capability in dealing with rare diseases. We have not experienced a Category 1 disease since 1999. Several people have been tested for VHF in the National Institute of Infectious Diseases of Japan every year, and all were negative [7]. Even under the raised screening sensitivity at quarantine in response to this large outbreak in West Africa, only nine cases were screened for EVD. A research group funded by the MHLW developed training programmes for staff in Class 1 hospitals, but only three-quarters of hospitals participated during 2011–13 [17]. In addition, the fact that only 29 of 45 Class 1 hospitals have infectious disease specialists qualified by the Japanese Association for Infectious Diseases, indicates that Class 1 hospitals have not played their expected role as core hospital for infectious diseases in the regions. Although any prefecture should be prepared for the appearance of highly infectious and pathogenic diseases with an appropriate level of biosafety, a more centralised strategy would more effectively concentrate available expertise and capacity for treatment of such extremely rare diseases in a sustainable manner.

The next important step would be to further improve practices in designated hospitals such as infection control measures in a biosafety ward. Specified hospitals should have a role in liaising with Class 1 hospitals to provide expertise, training, and simulation exercises. The legacy was the establishment of the Expert Committee for Treatment of Category 1 Diseases at the MHLW in October 2014. The Committee discussed therapeutic protocols, including the use of unapproved treatments. This platform will facilitate the sharing of up-to-date knowledge and of limited expertise for treatment and management of such rare diseases, and will support clinicians who care for patients with Category 1 diseases.

Emergency use of medical countermeasures

Favipiravir (Toyama Chemicals Co., Japan) is a drug licensed in Japan and indicated for infections with novel or re-emerging influenza viruses, although its use is limited to cases in which other anti-influenza virus drugs are ineffective or not sufficiently effective. Favipiravir is not yet marketed, and can only be distributed by order of the MHLW in the case of an emergency in an influenza pandemic. Although Favipiravir is not approved for EVD, it is expected that it can be used for EVD, as its efficacy for EVD has been shown in a mouse model [18]. There was a stock for 20,000 courses in tablet form, and for roughly 300,000 courses in active ingredients, in influenza treatment doses, in the company, as at 20 October 2014 [19].

Japan does not have an official access programme for use of unlicensed drugs outside clinical trials. The official view of the GOJ was that 'use of an unlicensed drug at a physician's discretion may be allowable' [20] and 'may not violate the Act on Pharmaceutical Affairs in this emergency situation' [21]. There remains a concern that there is no monitoring scheme for the efficacy and safety of unlicensed drugs, and no framework for an ethical consideration of their use. In response, the Expert Committee for Treatment of Category 1 Diseases recommended on 24 October 2014 that clinical data should be collected and shared with the public on the use of an unapproved drug [22]. A clinical research protocol for use of unapproved treatments in a Specified hospital was formulated. Such a framework prepared in advance should help provide the best emergency therapeutic options in an ethical manner, and track efficacy and safety of treatments for future emerging diseases without approved treatment options.

Conclusions

The EVD outbreak in West Africa provided a crucial opportunity to reveal challenges and improve preparedness for managing rare but high impact emerging diseases that are prone to be neglected. MHLW held a review meeting on the response to the EVD outbreak in West Africa to develop a technical guidance on preparedness and response to VHFs for public health agencies in October 2015 [23]. Some measures such as the case management policy may be country-specific; however, some challenges may not be specific to our country; for example, the strategy for sharing medical response capacity is a common concern for other countries, regions or at global level. Efforts to maintain achievements in public health preparedness as legacies may help contain future emerging diseases, including acts of bioterrorism and pandemic influenza.

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Conflict of interest

None declared.

Authors' contributions

Tomoya Saito performed the investigation and drafted the manuscript.

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Mobile diagnostics in outbreak response, not only for Ebola: a blueprint for a modular and robust field laboratory

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We established a modular, rapidly deployable laboratory system that provides diagnostic support in resource-limited, remote areas. Developed as a quick response asset to unusual outbreaks of infectious diseases worldwide, several of these laboratories have been used as part of the World Health Organization response to the Ebola virus outbreaks by teams of the 'European Mobile Lab' project in West Africa since March 2014. Within three days from deployment, the first European mobile laboratory became operational at the Ebola Treatment Unit (ETU) in Guéckédou, southern Guinea. Deployment in close proximity to the ETU decreased the turnaround time to an average of 4 h instead of several days in many cases. Between March 2014 and May 2015, more than 5,800 samples were tested in this field laboratory. Further EMLab units were deployed to Nigeria, Liberia and Sierra Leone in the following months of the Ebola outbreak. The technical concept of the EMLab units served as a blueprint for other mobile Ebola laboratories which have been set up in Mali, Côte d'Ivoire, Sierra Leone and other countries in West Africa. Here, we describe design, capabilities and utility of this deployable laboratory system for use in response to disease outbreaks, epidemiological surveillance and patient management.

Background

Since December 2013, West Africa has been facing the deadliest outbreak of Ebola virus disease (EVD), previously known as Ebola haemorrhagic fever. The current epidemic has killed within a year of its onset more than 10,300 people as reported from eight affected countries Liberia, Guinea, Sierra Leone, Mali, Nigeria, Spain, the United Kingdom and the United States [1]. It was as early as March 2014 that two European laboratories identified the causative agent as the *Zaire ebolavirus* [2], which was immediately followed by a response of the World Health Organisation (WHO) with the support of its Global Outbreak Alert and Response Network (GOARN).

EVD usually begins with fever, chills, and general malaise. These and other symptoms, including fatigue, headache, vomiting, diarrhoea, anorexia, and myalgia [3], can also result from a number of other infectious diseases endemic in Africa, e.g. malaria, dengue, leptospirosis and typhus [4]. Therefore, accurate identification of the causative agents is critical for effective containment of the outbreaks and provision of appropriate supportive care to the patients [5-9]. In preparedness for rapid, global response to naturally occurring and emerging infectious disease outbreaks, the International Cooperation and Development Office of the European Commission (DG DevCo) launched the European Mobile field laboratory (EMLab, www.emlab.eu) initiative in 2012. Three mobile laboratory units were established in home bases in Europe (Munich, Germany), West Africa (Nigeria) and East Africa (Tanzania).

The concept of a modular, rapidly deployable field laboratory was previously developed at the Bundeswehr Institute of Microbiology in 2008. The primary goal of the Bundeswehr Medical Mobile Laboratory (BML) initiative was to establish field-deployable diagnostic capabilities for medical surveillance and investigation of unusual disease outbreaks in theatres of military operation. The driving design criterion was to enable

Figure 1

Mobile Field Laboratory equipment in different phases of deployment

A. Airlift of a complete BML as passenger luggage, including two rapidly inflatable tent systems.

B. EMLab consortium upon arrival in Guéckédou, Guinea in March 2014.

C. Improved glovebox unit for sample inactivation.

D. First EMLab team providing Ebola diagnostics in a tent inside the MSF treatment centre in Guéckédou, Guinea, April 2014

worldwide deployment within 72 hours with minimal logistical burden (Figure 1). Therefore, modular units with multiple, flexible configurations to accommodate different mission requirements were envisioned. The BML is staffed with a four-person team equipped with the capability to provide confirmatory identification of a variety of bacterial and viral pathogens and biological toxins [10]. From 2008 to 2014, the BML has successfully been deployed in several missions to the Balkans, South and Central Asia, West Africa, North America and Europe (data not shown). Based on the BML model, the EMLab units were adopted for civilian multinational outbreak response missions in Africa and Europe.

On 26 March 2014, an EMLab team was deployed to Guinea at the request of the Guinean Ministry of Health and WHO GOARN. Within three days, the first mobile laboratory became operational at the Ebola Treatment Unit (ETU) in Guéckédou, southern Guinea, and its impact was evident as the turnaround time from specimen collection to reporting of test result was reduced from days to hours. Between March 2014 and May 2015, more than 5,800 samples were tested in this field laboratory (Table 1). Further EMLab units were deployed to Nigeria, Liberia and Sierra Leone in the following months of the Ebola outbreak. In addition, the technical concept of the EMLab units served as a blueprint for other mobile Ebola laboratories which have been set up in Mali, Côte d'Ivoire, Sierra Leone and other countries in West Africa.

Key elements of mobile laboratory units

Four elements were identified as essential for effective mobile laboratories: (i) personnel and training, (ii) biosafety and biosecurity management, (iii) methods and equipment and (iv) logistical support. These elements can be adapted to suite specific missions according to the nature of outbreaks, geographic areas, season and climatic conditions, and on-site resources.

Personnel and training

The human factor is the most critical element for a successful mobile field laboratory mission. Modern laboratory equipment and sophisticated diagnostic methods have to be handled by highly skilled laboratory personnel in the environment of a field laboratory. Previous experience in biosafety level (BSL) 3 or BSL-4 laboratory work can facilitate, but cannot replace, the adaption of the laboratory personnel to the biosafety and biosecurity procedures of a field laboratory. We consider a four-person team as the ideal size for fieldwork over a period of three to six weeks to allow for rest and rotation during peak operations. Handover from one team to the next should consider an overlap of at least two days to ensure seamless transition and continuation of the operation.

Before deployment, all team members should be trained under realistic field conditions. Based on the experience of several laboratory deployments, we developed a training curriculum consisting of 25 modules. It covers realistic scientific, medical, technical and operational challenges that could be encountered in a field situation. A shortened version of this training enables participants to conduct Ebola diagnostics in one of the already established EMLab units in West Africa during the latest outbreak (Table 2).

Biosafety and biosecurity management

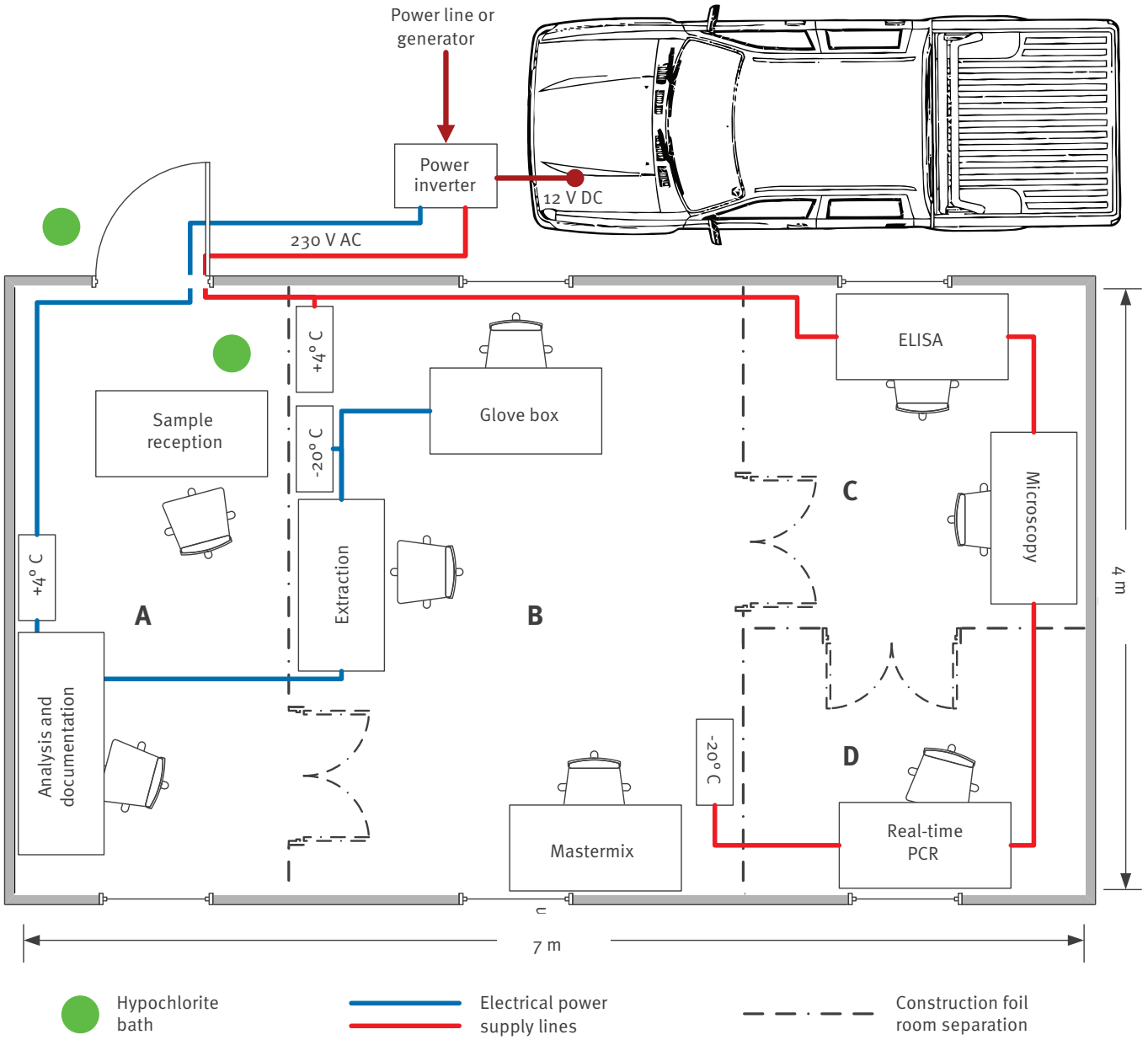
The difficulty of operating and relocating BSL-3 or BSL-4 laboratories in the field environment is well understood [11]. Several container-based or truck-mounted laboratory units have been purported to meet most BSL-3 regulatory requirements. However, those have rarely been deployed worldwide due to their large footprint, heavy weight and high logistical burden.

These constraints often interfere with rapid and timely response because sophisticated air, maritime and land transport infrastructure are needed as well as significant and continuous technical maintenance. Most of these requirements cannot be met in rural areas of developing countries, such as Guinea, Sierra Leone or Liberia.

In general, biosafety in a mobile laboratory is achieved by optimising laboratory practices, equipment safety and qualification of the laboratory staff [12-14]. For example, neither cultivation nor enrichment of

Figure 2

Schematic setup of the European Mobile Field Laboratory equipment and layout of two independent electrical supply lines in a tent or a fixed building



Hypochlorite baths in front of the field laboratory and at the sample reception are used to disinfect the packaging of submitted samples before further processing in the laboratory.

infectious material are foreseen in the mobile laboratory as this would dramatically increase biosafety risks as well as biosecurity risks [15]. The diagnostic procedures used, e.g. qPCR or RT-qPCR, immunoassays or light microscopy, do not depend on living organisms, and the specimens can easily be inactivated by validated chemical or heat treatment methods before further analysis [16-18].

For the protection of laboratory personnel during sample inactivation, two options can be employed: One option is wearing personal protective equipment (PPE), e.g. coverall suits, goggles and FFP-3 face masks within a designated 'hot zone' [6]. However, this option has a number of disadvantages: (i) the time laboratory personnel can work in full protective gear is very limited especially in tropical climate, (ii) a designated separate 'hot zone' is needed, adding another factor to the already complex task of setting up a field laboratory, and (iii) this 'hot zone' has to be considered as contaminated until it has been completely disinfected. The second option is the use of a hermetically sealed glovebox system. Gloveboxes are regarded as the best way to minimise the risk of exposure for workers especially

Table 1

European Mobile Laboratory mission statistics, as per 31 October 2015

EMLab (country)	EMLab (town)	First sample tested	Last sample tested	Operation time (weeks)	Number of teams	Samples tested	EBOV-positive (qPCR)
Guinea	Guéckédou	30 Mar 2014	3 Apr 2015	53	15	5,842	2,231
Guinea	Coyah	17 Feb 2015	Ongoing	30	9	5,292	487
Nigeria	Enugu and Port Harcourt	20 Aug 2014	1 Oct 2014	5	2	30	3
Liberia	Foya	13 Sep 2014	4 Dec 2014	12	4	315	81
Sierra Leone	Freetown and Kambia	14 Dec 2014	Ongoing	46	9	4,220	287
Sierra Leone	Hastings	22 Dec 2014	5 Sep 2015	43	10	4,012	343
Total					**49**	**19,711**	**3,432**

EBOV: Ebola virus; EMLab: European 'Mobile field laboratory'.

in hot climate. At the same time, heat stress is reduced for the operator since the glovebox renders the PPE dispensable. Only in case of failure of the glovebox, the laboratories teams would rely on PPE (coveralls and powered air-purifying respirators with hoods) for sample inactivation. In addition, the PPE is to be used to safely unpack samples that are too large for the glovebox or in the unlikely event of contamination inside the mobile laboratory environment.

Several flexible film isolator models have previously been used as gloveboxes in field laboratory missions [5,19]. Although designated as portable, we found that those units are either too large and heavy to be easily deployed, or, because non-solid walls are used, only minimal negative pressure can be applied to the interior of these gloveboxes. Negative pressure of more than –1 mbar (–2 mbar is the minimum requirement for stationary BSL-3 glovebox systems [20]) would lead to the collapse of the flexible film walls.

We bridged the gap between biosafety needs and mobility by developing a foldable glovebox system with solid polycarbonate walls (Figure 1C). Its design was based on the basic technical requirements for microbiological safety cabinets described in European Standard EN 12469 [20]. With a negative internal pressure up to –2.5 mbar that can be monitored and a leak rate less than 10% per 30 min at an overpressure of 5 mbar, this glovebox combines the safe working environment of a stationary glovebox with minimised packing size. Uncomplicated and safe handling was the main objective for the design of this foldable glovebox system. In the course of the Ebola missions in West Africa, the glovebox design was further optimised for durability and resistance in hot climate. The gloves can be adapted to the individual hand size of the operator to allow comfortable and safe performance of the inactivation protocols. Damaged gloves or sleeves can easily be replaced aseptically during glovebox operation. Two rapid transfer ports of different size, a well-established docking system in the pharmaceutical industry, allow contamination-free, safe and easy transfer of samples, supplies and waste into and out of the glovebox without breaking the containment.

All patient samples are stored in secondary packaging (plastic tubes or bags labelled with bleach resistant markers), floating in buckets filled with hypochlorite until further processing. This ensures thorough surface decontamination. For protection of the laboratory staff, all patient samples are finally unpacked inside the glovebox only. Two field laboratory team members operate the foldable glovebox: One operator is conducting all processing steps in the glovebox while another team member crosschecks and documents all steps with biosafety or diagnostic relevance on the respective laboratory request form. To prevent cross-contamination, samples are inactivated one after another, not simultaneously.

Methods and equipment

The BML system offers a range of diagnostic technologies, including qPCR, ELISA, immunofluorescence assays (IFA), immunochromatographic tests and microscopy. It is important to emphasise that all assays have to be adapted to and validated on the laboratory equipment before being included in the diagnostic portfolio of the mobile laboratory. Over the past seven years, we established more than 50 assays for 33 different pathogens and toxins that can be used under field conditions with the BML system. Performance of all PCR assays is controlled by the use of either competitive or non-competitive internal controls (IC), as well as extraction, negative and positive controls. Generally, IC are added to lysis buffer and patient serum samples before nucleic acid extraction in order to validate the complete diagnostic process.

For the ongoing Ebola outbreak in West Africa, we decided to focus on the detection of Ebola virus in different body fluids using RealStar Filovirus Screen RT-qPCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany) and of Malaria parasites in the blood of

Table 2

Modular (71 h) training modules for preparation of mobile laboratory teams

Introduction in the mobile field laboratory concept	**1 h**
Field laboratory setup	3 h
Technical instruction on handling of mobile laboratory equipment	**2 h**
Immunofluorescence and light microscopy protocols	2 h
Glovebox maintenance	**1 h**
SmartCycler maintenance and repair	1 h
Personnel protective equipment	**2 h**
Power generator operation and maintenance	1 h
Power converter operation	**1 h**
Scenario-based laboratory team training	**24 h**
Additional scenario-based team training	8 h
Sample reception and documentation	**1 h**
Documentation, statistics and reporting of data	2 h
qPCR results interpretation and troubleshooting	**2 h**
Gel electrophoresis	2 h
Storage of samples	**1 h**
Preparation of samples for IATA shipment	2 h
Satellite phone communication	**1 h**
VHF radio communication	2 h
Map and GPS training	2 h
Team safety and security in the field, including mission specific sociocultural aspects	**2 h**
Advanced laboratory troubleshooting	2 h
Disinfection regime and waste management	**2 h**
Additional laboratory waste management in the field	1 h
Field laboratory dismantling and packaging	3 h

GPS: global positioning system; IATA: International Air Transport Association; VHF: very high frequency.

Training modules printed in **bold** are included in the condensed (40 h) training designed to handle demand for the Ebola outbreak support mission in 2014 and 2015.

the patients (BinaxNow, Alere, Cologne, Germany). The Filovirus RT-qPCR kit includes an IC that allows to check for RT-PCR inhibition. For a valid negative result, the IC and the positive control must show a signal at the correct cycle threshold (Ct) value, and the extraction control must not show amplification. Each run was validated independently by two team members. During the EMLab missions in West Africa, ca 10% of all runs did not meet these criteria, mainly due to IC failure, and had to be repeated. Additional tests were performed (at the request of the sender) for Ebola virus-negative cases, including West African Lassavirus-specific qPCR, Dengue immunochromatographic tests, Ebola IgG IFA and Malaria microscopy. In the course of the Ebola outbreak in West Africa, EMLab teams have successfully participated in two independent laboratory quality control trials conducted by WHO and the European Network for Diagnostics of Imported Viral Diseases (ENIVD).

Turnaround time is an important indicator of mobile laboratory performance, and can be influenced by many factors, including sample collection, transportation, preparation, analysis, result interpretation and reporting. Time for transportation of samples to a laboratory is the factor contributing most to the total turnaround time. Deployment of the first EMLab unit in close proximity to the ETU in Guéckédou decreased the turnaround time to an average of 4 h instead of several days in many cases. A typical 12 h workload for a four-person laboratory team was 40 samples. The glovebox-based sample inactivation may present a bottleneck. However, well-trained laboratory teams should be able to process up to 90 samples within 12 hours as demonstrated by several EMLab teams during the peak of the Ebola outbreak in Guinea.

The molecular diagnostics capability of the EMLabs is primarily based on a ruggedised and robust qPCR instrument (SmartCycler, Cepheid, Sunnyvale, CA) with real-time PCR modules than can be programmed independently, which has previously been used in several field laboratory missions [5,6,21,22]. Each instrument is able to test up to 16 different samples in parallel using different thermocycling conditions, and a new run can always be started while other runs are still in progress. In contrast to other qPCR instruments, the optical system has no moving parts or bulbs that could be damaged during transport. In case of technical failure of one of the 16 PCR modules the remaining modules are still operational. Furthermore, laboratory team members can easily replace the defective modules in the field.

Given the fact that in remote regions, replacement of broken or lost equipment can be difficult or impossible within a reasonable time frame, a concept of fallback options for essential parts of the field laboratory has been implemented: Key components such as centrifuges, power sources and IT equipment are safeguarded against failure by duplication. If the qPCR cycler would be irrevocably damaged, small conventional thermal cyclers and gel electrophoresis systems are available.

The infrastructural requirements for setting up the mobile laboratory are minimal. Generally, almost every local building can be used as long as it provides at least 28 m² of space. In several overseas deployments, the rapidly inflatable BML tent system with prefabricated interior room separations (ADS 1611 B-LABORATORY, OWR, Elztal-Rittersbach, Germany) was successfully used even in remote areas. Without the need for a dedicated 'hot room' for sample inactivation, the mobile laboratory requires a minimum of two rooms, to separate the pre- and post-PCR area (Figure 2 A/B and C/D). However, subdivision of the laboratory into four rooms (Figure 2 A–D) is preferred and can be prepared by the team using construction foil, foil doors and duct tape. The complete set-up of the field laboratory takes 4–5 h to operational readiness. The laboratory design adopts

Table 3 A

Complete list of components of the European Mobile Field Laboratory

Module/items			
Biosafety protective equipment 3 packages, 72 kg			
Rapid Containment Kit Ver 03, OWR Rittersbach	2	Rapid Containment Kit, accessories, set 'Munich'	1
Laboratory personal protective equipment 2 packages, 36 kg			
Single-use protective suit, class 3b	10	Hose for blower unit	4
Hood, 3M Versaflo S-333G	6	Adapter plug	1
Blower unit, Micronel C420	4	Adhesive tape	1
Cooling vest, TST Sweden	4	Apron, single-use	15
Laboratory safety googles	6	Battery charger for blower unit	1
Nitrile gloves, long sleeves, large	20	Rechargeable batteries for blower unit	6
Nitrile examination gloves, medium	20	Rubber boots	5
P3 RD filter canister	12	FFP-3 respirator mask, single-use	15
Pen	5	Butyl gloves	6
Sample preparation 3 packages, 47 kg			
Centrifuge, Hettich EBA 21	1	Bio bottle, class 6.2 packaging	6
Centrifugation tube, polycarbonate, 50 ml	12	Scissors, single-use	10
Paper wipes, pack	10	Gaiter, pack	2
Pipette, 100 µL	1	Disposable plastic sharps container	2
Pipette, 1,000 µL	1	Forceps, single-use	5
Plastic bottle 1 L, scaled	3	Dengue rapid test	30
Polyethylene construction sheeting, 20 m²	1	HIV 0.5 rapid test	30
Ring adaptor for gloves	2	Influenza A and B rapid test	30
Tape	1	Malaria rapid test	500
Tube, cone-shaped, 50 mL	20	Nitrile examination gloves, large	30
Warning sign 'biohazard'	6	Nitrile examination gloves, medium	50
Waste bags, autoclavable	20	Nitrile examination gloves, small	50
Pre-PCR 2 packages, 57 kg			
Centrifuge, Hettich EBA 21	1	Adhesive towel drape	10
Centrifuge, VWR Galaxy mini	1	Autoclave tape	1
Mastermix glovebox, Captair Pyramide	2	Reaction tube, 2 mL	200
QIAamp DNA blood and tissue mini kit	1	Cooling block	2
QIAamp stool kit	1	Filter tips, 10 µL, pack	4
QIAamp viral RNA kit	1	Filter tips, 100 µL, pack	4
Thermomixer	1	Filter tips, 1,000 µL, pack	5
Vortex mixer	1	Laboratory coat, single-use	6
Parafilm, pack	1	Laboratory timer	1
SmartCycler PCR tubes	500	Micro test tube, 1.5 mL	200
Pen, alcohol resistant	2	Micro test tube, 1.5 mL sterile	100
Phosphate-buffered saline tablets, pack	10	Paracord, 20 m	1
Pipette, 10 µL	2	Tube rack, 0.2 mL	1
Pipette, 100 µL	3	Tube rack, 1.5 mL	4
Pipette, 1,000 µL	2	Tube rack, 50 mL	1
Polyethylene construction sheeting, 20 m²	1	Tube, cone-shaped, 50 mL	25
Syringe, 10 mL	50	Storage box	5
Syringe filter, 0.2 µm	50		
Real-time PCR 2 packages, 60 kg			
Real-time PCR, SmartCycler II	1	iCore modules for SmartCycler	3
PCR termal cycler, Finnzyme Piko	1	Autoclave tape	1
Parafilm, pack	1	Centrifuge, small	1
Pen, alcohol resistant	10	Disposable plastic sharps container	2
Pipette, 10 µL	1	Filter tips, 10 µL, pack	4
Pipette, 100 µL	1	Filter tips, 100 µL, pack	4
Pipette, 1,000 µL	1	Filter tips, 1,000 µL, pack	4
Polyethylene construction sheeting, 20 m²	1	Gaiter, pack	2
Reaction tube	200	Laboratory notebook	2
Tube rack, 0.2 mL	1	Laboratory coat, single-use	6
Tube rack, 1.5 mL	2	Nitrile examination gloves, large	70
Vortex mixer	1	Nitrile examination gloves, medium	30
Waste bags, autoclavable	25	Nitrile examination gloves, small	40

Table 3 B

Complete list of components of the European Mobile Field Laboratory

Module/items			
Post-PCR 1 package, 29 kg			
Gel electrophoresis, Lonza FlashGel	1	Laboratory timer	1
FlashGel cassette 2.2%	9	Microtitre plate	10
Electrophoresis power supply, 300 V	1	Pen, alcohol resistant	2
Nitrile examination gloves, large	70	Pipette, 10 µL	1
Nitrile examination gloves, medium	30	Pipette, 100 µL	1
Nitrile examination gloves, small	40	Pipette, 1,000 µL	1
Adhesive towel drape	4	Plastic bottle 1 L, scaled	2
Autoclave tape	2	Polyethylene construction sheeting, 20 m²	1
Disposable plastic sharps container	1	Purified water, infusion bag, 500 mL	4
Filter tips, 10 µL, pack	3	Tube rack, 0.2 mL	1
Filter tips, 100 µL, pack	3	Tube rack, 1.5 mL	2
Filter tips, 1,000 µL, pack	4	Vortex mixer	1
Gaiter, pack	2	Waste bags, autoclavable	20
Laboratory coat, single-use	6	Micro test tube, 1.5 mL, sterile	200
ELISA 1 package, 24 kg			
ELISA reader, TECAN Sunrise	1	Adhesive seals, pack	1
Mini-incubator (37 °C), HumaTemp	1	Combitip dispenser	2
Multi-channel pipette 100 µL	1	Combitips, 10 mL	30
Multi-channel pipette 300 µL	1	Combitips, 1 mL	40
Paper wipes, pack	10	Microtitre plate	10
Phosphate-buffered saline tablets	100	Filter tips, 100 µL, pack	5
Pipette, 100 µL	1	Graduated pipette, single-use	10
Plastic bottle 1 L, scaled	2	Laboratory timer	1
Microscopy 1 package, 31 kg			
Microscope, Partec CyScope	1	Cover glass	100
Polyethylene construction sheeting, 20 m²	1	Disposable plastic sharps container	1
Purified water, infusion bag, 500 mL	3	Glass slides	100
Staining solution, Gram	1	Immersion oil, pack	1
Staining solution, McFadyean	1	Inoculating loop, pack	3
Storage box for microscope slides	2	Laboratory coat, single-use	8
Tube rack, 50 mL	2	Laboratory timer	1
Tube, cone-shaped, 50 mL	2	Pipette Pasteur, single-use	60
Tweezers, single-use	2	Nitrile examination gloves, large	30
Urine beaker with seal	2	Nitrile examination gloves, medium	20
Waste bags, autoclavable	5	Paper wipes, pack	10
Waste bottle 1 L, teflon	1	Pencil	1
Active cooling (-20 °C/4 °C) 2 packages, 50 kg			
Cooling box, CoolFreeze CFX 50, WAECO	2		
Passive transport cooling 2 packages, 40 kg			
Mini Vacuum Case, DeltaT	2	Cooling elements, DeltaT, –21 °C	10
Cooling elements, DeltaT, +4 °C	10	Diagnostic reagents according to mission requirements	
Documentation and communication 1 package, 31 kg			
Laptop, ruggedised, GETAC V100	2	Inkjet printer cartridges	3
Mobile document scanner	1	Office printer paper, pack	1
Mobile inkjet printer	1	Pen, document-proof	5
Compact camera, ruggedised, LUMIX DMC-FT5	1	Self-adhesive laboratory labels, numbered consecutively	500
Satellite phone, Thuraya XT	1	USB cable	2
Laboratory notebook	2	USB stick 4 GB, write-protectable	4
Field laboratory request forms	500	First aid kit	1
Electricity 2 packages, 50 kg			
Power inverter, 12–230 V, 1,500 W	2	5-fold socket, integrated overvoltage protection	6
Residual-current circuit breaker adapter plugs	2	Battery tester	2
Nitrile examination gloves, large	200	Battery connection cables for power inverter	2
Nitrile examination gloves, medium	200	Duct tape	2
Nitrile examination gloves, small	200	Extension cable, 10 m	2
Polyethylene construction sheeting, 20 m²	2	International socket adapter plug sets	2
Working place lights	4		
Total number of packages (each max. 31 kg weight) for complete mobile field laboratory deployment: 22 packages, 527 kg			

a strict one-way rule for processing of the samples, starting from the sample reception area (Figure 2A), to sample inactivation and nucleic acid isolation (B), ELISA and microscopy (C) and ending in the PCR analysis room (D) to avoid cross-contamination of the samples. In order to reduce the number of separated rooms, the preparation of the PCR master mix is located inside a flexible film isolator designed for material protection. Tables and chairs are not part of the mobile laboratory equipment and have to be acquired locally. They are also covered with construction foil in order to facilitate easy surface decontamination. If electrical power is available locally (from portable generators or power line), power inverters are used as back-up systems and interruption-free power supplies (Figure 2). Each laboratory is equipped with two 1,500 W power inverters that provide 230 V AC power from a car battery.

Logistical support

Robustness and transportability are key features of our mobile laboratory design. Therefore, all laboratory instruments are selected for size, weight and durability, even under extreme field conditions. The complete laboratory equipment is packed in wheeled, ruggedised, water- and dustproof boxes (Figure 1B). More than 400 items are allocated to 27 boxes (Table 3). Box weights ranging from 20 kg to a maximum of 31 kg allow not only their transport as passenger luggage by all international airlines, but also ease handling by the laboratory team during reloading and land transport. Although customs-related import problems are likely to be specific to every deployment, meticulous preparation of foreseeable transport documents (pro-forma invoice, import/export declaration forms, tax exemption certificates and multilingual packing lists) facilitates customs clearance significantly [23].

To guarantee a high degree of flexibility, different sets of equipment are packed for specific deployment missions. If not needed for a certain mission, individual boxes (e.g. for ELISA or microscopy) can be omitted from the deployment without affecting the performance of the other laboratory functions. To ensure the cleanliness of the consumables, such as gloves and reaction tubes, and minimise their space requirement in the boxes, they are packed in daily consumption quantities and vacuum-sealed in multilayer laminated aluminium foil pouches. Consumables and reagents are apportioned to allow continuous operation of a single laboratory for up to 50 days (based on the assumption of handling 25 samples per day) without logistic support. In the current EMLab missions, replacement teams replenish stocks of consumables. Since the active cooling or freezing devices of the laboratory cannot be operated during air transport, vacuum insulated boxes are used to bring temperature-sensitive reagents into the field.

Conclusions

The latest Ebola outbreak support mission, conducted by the EMLab consortium in several West African countries, has confirmed that rapid turnaround times are critical for surveillance and patient management, and at the same time facilitated unique scientific studies. Besides human diagnostics, the EMLabs provided high-quality laboratory expertise to support research and development activities for the assessment of new Ebola rapid diagnostics, for Ebola treatment trials and for Ebola vaccine research [24-26]. They were also engaged with other partners in operational research to review or confirm public health prevention and control strategies [27]. From March 2014 to October 2015, more than 19,000 samples were tested in the EMLab units. The overall costs for the complete field laboratory equipment described here are ca EUR 150,000, compared with the more than 10 times higher costs of other container- or truck-based solutions (DG DevCo, personal communication, 23 April 2015). Even though some security, safety and health risks are always present in mobile missions, they can be mitigated by careful planning, mission preparation and team training. With these measures, a safe working environment was ensured during more than 1,300 Ebola laboratory mission days in West Africa during the rainy season and the dry season.

The mobile laboratory concept described here is a forward-looking solution for outbreak response in remote areas where emerging infectious diseases might occur. It offers rapidly deployable, state-of-the-art technology for effective field diagnostics capabilities. Our field laboratory concept has served as a blueprint for similar projects and may continue to provide a model for future mobile laboratory projects.

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Conflict of interest

The corresponding author is listed as a co-inventor of the foldable glovebox system in a European patent. All other authors indicate no conflicts of interest.

Authors' contributions

RW designed the mobile field-lab concept. EF, BG, PM, LZ, GG, KS and RW developed, assembled and validated the mobile field-lab systems. KS and RW wrote the first draft of the paper. ADC, SG, SI, AOH, PF and LZ critically reviewed the

first draft of the paper, and their comments were incorporated. All authors read and approved the final manuscript.

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